EXPRESSION OF KI67, P53 AND MUC1 IN RENAL CELL CARCINOMA IN CORRELATION WITH NUCLEAR GRADE

Dissertation submitted in partial fulfilment
of the requirements for the degree of

M.D. (PATHOLOGY)
BRANCH - III

INSTITUTE OF PATHOLOGY,
MADRAS MEDICAL COLLEGE,
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THE TAMIL NADU
DR. M.G.R. MEDICAL UNIVERSITY
CHENNAI

APRIL 2015

CERTIFICATE

This is to certify that this Dissertation entitled **" EXPRESSION OF KI67, P53 AND MUC1 IN RENAL CELL CARCINOMA IN CORRELATION WITH NUCLEAR GRADE"** is the bonafide original work of **Dr.G.SARUMATHY,** in partial fulfillment of the requirement for M.D.,(Branch III) in Pathology examination of the Tamilnadu Dr.M.G.R Medical University to be held in April 2015.

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DECLARATION

I **Dr.G.SARUMATHY,** solemnly declare that the dissertation titled **"EXPRESSION OF KI67, P53 AND MUC1 IN RENAL CELL CARCINOMA IN CORRELATION WITH NUCLEAR GRADE"** is the bonafide work done by me at Institute of Pathology, Madras Medical College under the expert guidance and supervision of **Prof. Dr. RAJAVELU INDIRA, M.D.,** Professor of Pathology, Regional Institute of Ophthalmology and GOH, Madras Medical College. The dissertation is submitted to the Tamilnadu Dr.M.G.R Medical University towards partial fulfillment of requirement for the award of M.D., Degree (Branch III) in Pathology.

Place : Chennai

Date : **Dr.G.SARUMATHY**

ACKNOWLEDGEMENT

I express my sincere thanks to **Prof. Dr. R. VIMALA, M.D.**, Dean, Madras Medical College and Rajiv Gandhi Government General Hospital, for permitting me to utilize the facilities of the Institution.

I take this opportunity to express my heartfelt sincere gratitude to **Prof. Dr. M. SARASWATHI, M.D.,** Professor and Director of Institute of Pathology, Madras Medical College, Chennai, for her constant encouragement, wholehearted support, valuable suggestions and expert guidance throughout the study, without which this study would not have ever been possible.

I am extremely thankful to **Prof. Dr. RAJAVELU INDIRA, M.D.,** Professor of Pathology, Regional Institute of Ophthalmology, Madras Medical College, for her valuable suggestions, constant support, advice and encouragements throughout the study

I am thankful to **Prof. Dr. P.KARKUZHALI, M.D.,** Professor and former Director of Institute of Pathology, Madras Medical College for her initial guidance and valuable suggestions during the study.

I am truly thankful to **Prof. Dr. SHANTHA RAVISANKAR M.D. D.C.P, Prof. Dr. R. PADMAVATHI M.D. D.G.O., Prof. Dr. V. RAMAMURTHY M.D., Prof. Dr. GEETHA DEVADAS M.D., D.C.P.,**

Prof. Dr. M. P. KANCHANA M.D., Prof. Dr. K. RAMA M.D., Prof. Dr. Prof. Dr. SUDHA VENKATESH M.D., and **Prof. Dr. S. PAPPATHI M.D., D.C.H** for their valuable suggestions and encouragement throughout the study.

I express my heartfelt sincere thanks to all my Assistant Professors for their help and suggestions during the study.

I would like to thank the Institutional Ethics Committee for approving my study. I am thankful to my colleagues, friends, technicians and staff of the Institute of Pathology , Madras Medical College, Chennai for all their help and support they extended for the successful completion of this dissertation .

Above all I thank the Lord Almighty for His kindness and benevolence without which this study would not have materialized.

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

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CERTIFICATE OF APPROVAL

To
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Dear Dr. G. Sarumathy,

The Institutional Ethics Committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled **"Expression of Ki67, P53 and MUC1 in renal cell carcinoma in correlation with nuclear grade"** No.04042014

The following members of Ethics Committee were present in the meeting held on 08.04.2014 conducted at Madras Medical College, Chennai-3.

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We approve the proposal to be conducted in its presented form.

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The Institutional Ethics Committee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and patients information / informed consent and asks to be provided a copy of the final report.

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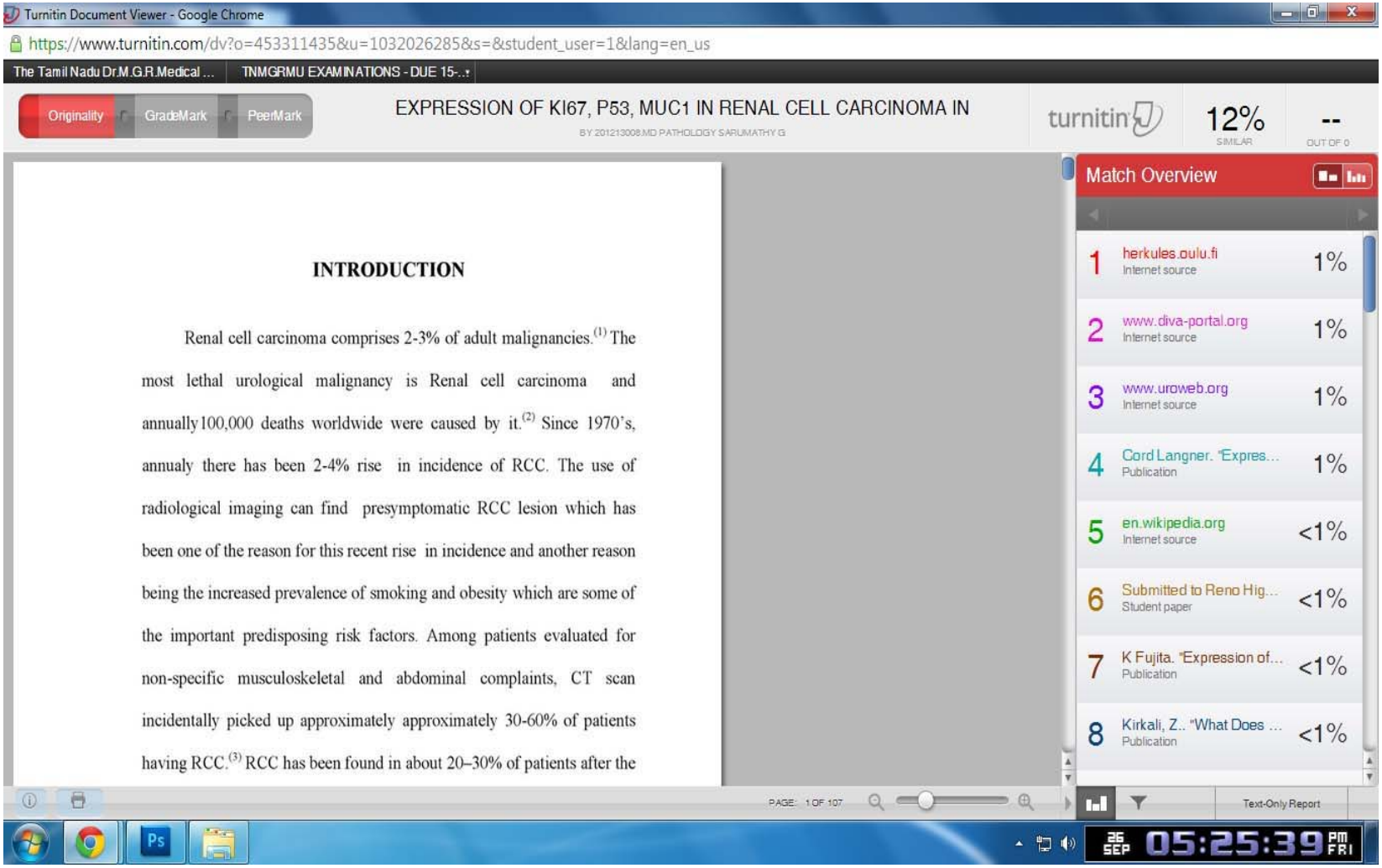
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INTRODUCTION
Renal cell carcinoma comprises 2-3% of adult malignancies.(1) The most lethal urological malignancy is Renal cell carcinoma and annually100,000 deaths worldwide were caused by it.(2) Since 1970's, annualy there has been 2-4% rise in incidence of RCC. The use of radiological imaging can find presymptomatic RCC lesion which has been one of the reason for this recent rise in incidence and another reason being the increased prevalence of smoking and obesity which are some of the important predisposing risk factors. Among patients evaluated for non-specific musculoskeletal and abdominal complaints, CT scan incidentally picked up approximately approximately 30-60% of patients having RCC.(3) RCC has been found in about 20–30% of patients after the
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File name:	C1_IN_RENAL_CELL_CARCINOMA...
File size:	135K
Page count:	107
Word count:	16,156
Character count:	84,839
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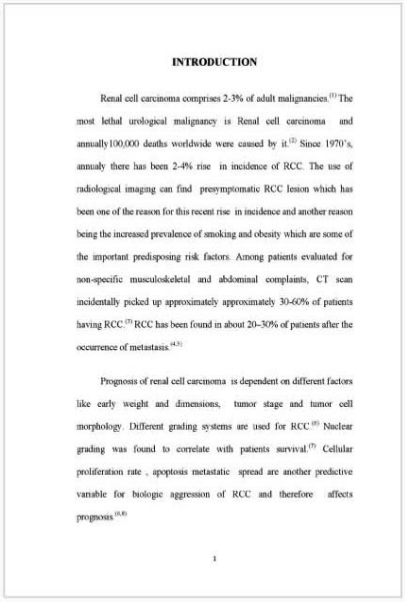
Submission ID:	453311435



INTRODUCTION

Renal cell carcinoma comprises 2-3% of adult malignancies.[1] The most lethal urological malignancy is Renal cell carcinoma and annually100,000 deaths worldwide were caused by it.[2] Since 1970's, annualy there has been 2-4% rise in incidence of RCC. The use of radiological imaging can find presymptomatic RCC lesion which has been one of the reason for this recent rise in incidence and another reason being the increased prevalence of smoking and obesity which are some of the important predisposing risk factors. Among patients evaluated for non-specific musculoskeletal and abdominal complaints, CT scan incidentally picked up approximately approximately 30-60% of patients having RCC.[3] RCC has been found in about 20–30% of patients after the occurrence of metastasis.[4,5]

Prognosis of renal cell carcinoma is dependent on different factors like early weight and dimensions, tumor stage and tumor cell morphology. Different grading systems are used for RCC.[6] Nuclear grading was found to correlate with patients survival.[7] Cellular proliferation rate , apoptosis metastatic spread are another predictive variable for biologic aggression of RCC and therefore affects prognosis.[8,9]

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ABBREVIATIONS

RCC	-	Renal Cell Carcinoma
PcNA	-	Proliferate cell nuclear antigen
VHL	-	Von Hippel-Lindau
CT Scan	-	Computed tomography
MRI	-	Magnetic Resonance Imaging
FNA	-	Fine Needle Aspiration
WHO	-	World Health Organisation
H & E	-	Hemotoxylin and Eosin
IHC	-	Immunohistochemistry
CK	-	Cytokeratin
EMA	-	Epithelial Membrane Antigen
VEGF	-	Vascular Endothelial Growth Factor
LI	-	Labeling Index
MIB – 1	-	Monoclonal antibody directed against Ki-67 protein
PCR	-	Polymerase chain reaction

CONTENTS

EXPRESSION OF KI67, P53 AND MUC1 IN RENAL CELL CARCINOMA IN CORRELATION WITH NUCLEAR GRADE

ABSTRACT

AIMS AND OBJECTIVES:

Renal cell carcinoma comprises 2-3% of adult malignancies. It has been very challenging to predict the prognosis of each of the patients with RCC; when assessing cancer prognosis, classic prognostic factors, staging and grading were also not always accurate in prediction. In different studies, Ki67, p53 and MUC1 have been considered as a good predictive marker for RCC aggression, prognosis and survival outcome of patients. In this study, an attempt has been made to compare the expression of Ki67, p53 and MUC1 markers with nuclear grade and other clinicopathological parameters.

MATERIALS AND METHODS:

The clinical and pathological findings of Renal cell carcinoma cases were retrieved from the pathology records from august 2011 to august 2014 in Rajiv Gandhi Government General Hospital, Chennai. Totally 52 cases of renal cell carcinoma was studied and of this, 40 cases were randomly selected and immunohistochemistry was done using Ki67, p53, and MUC1.

RESULTS:

Among 52 cases studied, 36 were clear cell RCC, 8 were papillary RCC, 2 were Chromophobe RCC, 6 were unclassified RCC. Most common nuclear grade was Furhman nuclear grade 3.

There was statistically significant association of Ki67 expression with nuclear grade and stage at presentation. Association between p53 and histological type was found to be significant. There is significant association of MUC1 expression with nuclear grade and stage.

CONCLUSION:

The combined detection of Ki67, p53 and MUC1 expressions, which are superior to single marker along with nuclear grade and stage, could be used to significantly improve the accuracy in predicting the prognosis of RCC patients.

KEY WORDS:

Renal cell carcinoma, nuclear grade, immunohistochemistry.

INTRODUCTION

Renal cell carcinoma comprises 2-3% of adult malignancies.[1] The most lethal urological malignancy is Renal cell carcinoma and annually100,000 deaths worldwide were caused by it.[2] Since 1970's, annualy there has been 2-4% rise in incidence of RCC. The use of radiological imaging can find presymptomatic RCC lesion which has been one of the reason for this recent rise in incidence and another reason being the increased prevalence of smoking and obesity which are some of the important predisposing risk factors. Among patients evaluated for non-specific musculoskeletal and abdominal complaints, CT scan incidentally picked up approximately approximately 30-60% of patients having RCC.[3] RCC has been found in about 20–30% of patients after the occurrence of metastasis.[4,5]

Prognosis of renal cell carcinoma is dependent on different factors like early weight and dimensions, tumor stage and tumor cell morphology. Different grading systems are used for RCC.[6] Nuclear grading was found to correlate with patients survival.[7] Cellular proliferation rate , apoptosis metastatic spread are another predictive variable for biologic aggression of RCC and therefore affects prognosis.[6,8]

Cellular proliferation rate in RCC could be evaluated by studying Ki67 antigen expression and PcNA (proliferate cell nuclear antigen). Apoptosis degree in tumor can be measured by detecting mutant P53 antigen.[6] MUC1 have a role in cellular polarity, cell adhesion, and signal transduction. In many epithelial cancers, there is a loss of polarized cellular expression and there is diffuse circumferential distribution. In carcinomatous cells ,these variations of expression are suspected to participate in the metastatic dissemination. [8]

It has been very challenging to predict the prognosis of each of the patients with RCC; when assessing cancer prognosis, classic prognostic factors, staging and grading were also not always accurate in prediction.[5,9] Treatment of metastatic RCC has dramatically changed in the last decade and leads to revival of new hope to patients affected by this malignancy and changed the traditional thinking of grave prognosis in terms of survival among patients diagnosed in advanced stages. So there has been a definite need for better tools in predicting the clinical course of RCC in this era of evergrowing novel molecular targeted therapies.

For proper counselling of the patient and for proper planning and individualizing patient treatment, accurate prognostication is of utmost importance.[9] In different studies, Ki67, P53 and MUC1 have been

considered as a good predictive marker for RCC aggression, prognosis and survival outcome of patients.[6,8]

The purpose of this study was to access the expression of P53, Ki 67, MUC1 in different types of renal cell carcinoma. The expression of these apoptotic, proliferative and metastatic marker was compared with the nuclear grading. Increasing severity and reactivity rate to these markers have been always followed by poor prognosis.

AIMS

AND

OBJECTIVES

AIMS AND OBJECTIVES

- To study the incidence and distribution of renal cell carcinoma in patients who attended Rajiv Gandhi Government General Hospital from august 2011 to august 2014.

- To study the clinicopathological features of renal cell carcinoma

- To determine the expression of ki67, P53 and MUC1 by immunohistochemistry in renal cell carcinoma.

- To study the correlation between ki67, P53 andMUC1 with nuclear grade

REVIEW OF LITERATURE

REVIEW OF LITERATURE

EPIDEMIOLOGY

Kidney tumor constitutes approximately 3% of all malignant tumors in adults.[2] Renal cell cancer (RCC) comprises 90% of all malignancies of the kidney that occur in adults in both sexes. Among males it ranks 6th in industrialized areas and 16th in less developed area. In women it ranks 12th in developed and 17th in developing countries respectively.[10] RCC is a heterogeneous group of hereditary or sporadic malignancies that arise from renal cells. Its frequency is next to prostate and bladder cancer, but it is the most lethal of these malignancies.

The incidence of RCC has been reported to be relatively high in North America, Scandinavia and Australia compared to other countries.[2] In several Eastern and Western European countries and also in parts of Italy, North America and Australia/New Zealand, incidence of RCC has been generally the highest. The lowest incidence of RCC are found in Africa and Asia.[11] The incidence of this malignancy has been increasing steadily at the rate of 2-3%per year.[12,13] Around 20-30% of the RCC has been estimated to present in the stage of metastasis.[14,15] It is also a well known fact that advanced overall TNM stage tumours can have low T-stage and they account for 25% of widely metastatic disease in few studies.[16]

AGE AND GENDER

Most commonly RCC occurs in the fourth to sixth decades of life, but both sporadic and in particular hereditary tumors have been reported in children.[17] Among both low and high risk countries, men were affected two to three times more commonly than women.[18]

RISK FACTORS

Most common cause of renal malignancy is tobacco smoking and in males, around 39% of all cases were caused by it.[19] Carcinogenic arsenic compounds in industrial processes or drinking water increases the risk by 30%.[20] Asbestos, cadmium, some organic solvents, pesticides and fungal toxins are addressed as possible carcinogens for the kidney but definitive evidence has not been established.[20,21]

Estrogens could be a risk factor for RCC in obese and overweight individuals. Several epidemiological studies conducted in many different populations have found out that the incidence of renal malignancy increases steadily with increase in body mass index (BMI).[22] Cho et al concluded in his prospective study that long term use of nonsteroidal anti-inflammatory drugs may raise the incidence of renal cancer.[23] In people suffering from chronic hypertension, the incidence of RCC is significantly increased that is independent of tobacco smoking and

obesity.[24,25,26] RCC has been established with exposure to analgesics containing phenacetin.[20]

CLINICAL PRESENTATION

According to the mode of detection RCCs are classified in two groups: symptomatic and incidental. The classic triad of presentation with hematuria, abdominal pain and flank mass is encountered less frequently than previously and is indicative of advanced disease. The typical tumor presents with lack of warning signs in early stage and can be clinically occult during majority of its time course. Majority of the RCCs are now incidentally found during investigations for complaints which are not expected in the renal cancer and due to the increasing use of imaging investigations such as ultrasonography(USG), computed tomography (CT)scan and magnetic resonance imaging (MRI) study.[27,28]

RCC remains a challenging malignancy due to its paraneoplastic manifestations such as hypercalcaemia, erythrocytosis, increased erythrocyte sedimentation rate, and non-metastatic hepatic dysfunction.

Most common presentations are

- Abdominal pain (40%)
- hematuria (40%)
- Abdominal mass (25%)

- loss of weight (33%)
- fever (20%)
- Systemic hypertension (20%)

Fatigue and varicocele, left side is usually affected, because of testicular vein` obstruction by the tumour extension(2% of males). The contribution of erythrocyte sedimentation rate (ESR) in prediction of prognosis has been a matter of debate in several studies.[29,30] However, in the recent studies of Kawai et al.[31] And Magera et al[32] preoperative ESR has been identified as a significant independent prognostic factor in patients with localized Clear Cell RCC. ESR is also found to be an independent prognostic factor in patients with metastatic RCC treated with or without cytoreductive radical nephrectomy.[33]

GENETICS

The Von Hippel-Lindau gene (VHL) is mutated or inactivated in most sporadic clear cell carcinomas which is an early event in the carcinogenesis of the tumor. The normal function of VHL includes regulation of oxygen dependent expression of genes which will regulate cellular response to hypoxia. These include genes associated with erythropoiesis, angiogenesis and resistance to hypoxia. Von Hippel-Lindau protein regulate ubiquitn –mediated destruction of hypoxia inducible factors. In the absence of VHLp there is high level of HIF

which induces production of endothelin-1, erythropoietin, vascular endothelial growth factor(VEGF), ceruloplasmin, transforming growth factor, cyclin G2, and these leads to tumor progression.[34]

Some genetic syndromes are associated with RCC. Von Hippel-Lindau disease is caused by mutation of Von Hippel-Lindau gene. This gene encodes a tumor suppressor protein. In 75% of sporadic clear cell carcinoma both gene copies are inactivated. Von Hippel-Lindau syndrome, or VHL disease, is syndrome with an autosomal dominant inheritance and it predisposes to a number of neoplasms, such as :
RCC having features of clear cell histology

- Pancreatic islet cell tumors and cysts
- Pheochromocytoma
- Hemangioblastomas
- Retinal angiomas
- Tumors of Endolymphatic sac
- Cystadenomas of epididymis.[35]

When compared to sporadic tumors these tumors occur in younger age. They are also commonly bilateral and multicentric.[36]

Hereditary leiomyomatosis and renal carcinoma

This is a syndrome with autosomal dominant inheritance and they occur due to germline mutation in fumarate hydratase gene.[37] This inherited disease causes the affected individuals to have an increased incidence to suffer from benign leiomyoma of skin and uterus and some of them develop RCC with type 2 features.[38]

Hereditary papillary RCC

Hereditary papillary RCC is one of the genetic disorder with a pattern of autosomal dominant inheritance; multifocal and bilateral papillary RCC occur in the individuals with this syndrome. MET protooncogene have been mutated in 85% of families[39]

TUBEROUS SCLEROSIS(TS)

This is a disorder of autosomal dominant pattern of inheritance . It is due to mutation in TSC1 or TSC2 gene. TSC1 is located on chromosome 9q34 .TSC2 is located on chromosome 6p13. Both of the genes encode tumor suppressor proteins. This disorder is characterised by multiple benign hamartoma in CNS, angiomyolipoma in kidney. There is an increase in incidence in RCC among these patients.[35]

BIRT-HOGG-DUBE SYNDROME

This syndrome has an autosomal dominant pattern of inheritance. These patients have a high predisposition to be affected by benign neoplasms of the hair follicle, colonic polyps and pulmonary cysts.. There is an increased incidence of renal tumors.[40]

HYPERPARATHYROIDISM (JAW TUMOUR SYNDROME)

Is a rare disorder with an autosomal dominant pattern of inheritance. Characterised by fibromas of the jaw, parathyroid adenoma and renal cell carcinoma.[41]

DIAGNOSTIC METHODS

The renal mass has a wide range of differential diagnosis and includes pseudotumors, benign cysts, angiomyolipomas, vascular malformations, Wilm's tumor, sarcoma, lymphoma, and metastases. However, percutaneous biopsy of a solid renal mass should not be undertaken, as more than 80% of such masses are RCC,[42] benign lesion and metastasis are rare .

As per the guidelines of National Comprehensive Cancer Network(NCCN) for the RCC management, patients with known or suspected renal cell carcinoma should be further evaluated with routine laboratory studies (chemistry panel, complete blood count, urine analysis,

partial thromboplastin time and prothrombin time,), abdomen and pelvis computed tomography(CT) scan, X-ray of the chest, and chest CT scan if the chest X-ray is abnormal or if there is extensive disease. Further studies such as a magnetic resonance imaging (MRI) of the brain and a bone scan should be undertaken only if clinically indicated.

CT or MRI

Renal mass can be usefully characterised by the MRI or CT scan of the abdomen. In most patients, RCC be accurately diagnosed by these imaging studies.

Information provided by abdominal CT are :

- Morphology and function of the opposite kidney[43]
- Anatomical extension of the primary tumour
- Venous extension / invasion;
- Regional lymph nodal involvement(enlargement) ;
- Involvement of the adjacent adrenal glands and metastasis toliver

Contrast-enhanced biphasic abdominal CT angiography can be of useful in surgical cases to obtain accurate information about the vascular supply of the affected kidney for on table clamping of segmental renal artery in cases planned for partial nephrectomy.[44,45] Biphasic MR angiography (MRA) can be done in the patient allergic to CT contrast but

MR angiography is less accurate than CT angiography in accurate depiction of accessory renal vessels .[46]

In patients with indeterminate CT results, MRI can give additional valuable information like :

- show any enhancing areas in renal masses (including enhancing septations, wall and nodular components in complex cystic masses).[47]
- more accurately establish the anatomical extensions in case of locally advanced malignancy.
- more accurately establish venous extension of the tumour, if the involvement of an inferior vena cava(IVC) tumour thrombus is poorly depicted on CT scan.[48,49]

MRI is also indicated in pregnant patients without renal failure.[50,51]

Among imaging for chest staging of metastatic RCC, most accurate investigation is the Chest CT. However, initially routine chest x-ray must be done for evaluation of large lung metastasis, although this is less accurate in finding small metastasis, when comparing CT chest. At the time of diagnosis, most brain and bone metastases are symptomatic and it is not generally advised to do routine bone or brain imaging in the further investigation of the known RCC patient.[52,53] However, CT brain, MRI

brain or bone scan, may be carried out in patients presenting with related clinical symptoms and signs.[54,55]

RENAL TUMOUR BIOPSY

Image guided percutaneous biopsies of renal tumour are of increasing use :

1. For histological diagnosis in case of radiologically indeterminate renal mass lesions;
2. For categorising patients into surveillance group, in case of small renal mass lesions;
3. To get histological diagnosis before proceeding onto ablative treatment procedures;
4. For deciding the most suitable means of targeted pharmacologic therapy for the stage of metastatic disease .[56,57,58]

Image guided percutaneous sampling in case of a renal mass can be done by means of trucut needle biopsy or fine needle aspiration. The main aim is to determine the nature of malignancy, its exact histological type, and its exact nuclear grade in view of its aggressiveness.

In view of the high accuracy in diagnosis of renal mass forming lesions by abdominal imaging, biopsy of the renal mass forming lesions

before surgery is not always necessary, as in case of healthy individuals having a long life expectancy.

Under local anesthesia, image guided percutaneous sampling in case of a renal mass can be done with the guidance of either ultrasound or CT.[59,60] Presently 18-gauge needles are considered ideal for trucut biopsies of the renal mass, as we can obtain sufficient amount of tissue and can be done with low morbidity for diagnostic purpose in the majority of cases undergoing percutaneous biopsies. The complications which we most frequently encounter in percutaneous biopsy of a renal mass are spontaneously resolving hematoma (perinephric /subcapsular) and hematuria; its unusual (0-1.4%) to encounter clinically significant bleeding after biopsy and is usually self-limiting .[56,60]

On comparing to FNA, trucut needle biopsies are usually more preferable in case of solid renal masses, in view of its higher diagnostic yield and higher accuracy rate. For detailed histopatholigical analysis of the malignancy, it is necessary to obtain at least two high quality biopsy cores (> 10 mm in length and non-fragmented) and to avoid sampling necrotic areas. In case of experienced biopsy centers, core needle biopsies of renal solid masses have obtain 78-97% diagnostic yield for the accurate diagnosis of the corresponding renal malignancies. However, it

must be kept in mind that 2.5-22% of trucut needle biopsies are non diagnostic.[61,62]

Tumour grade assessment on core biopsy specimen is very challenging. The obtainable accuracy of Fuhrman grading on trucut needle biopsies is poor (43-75%); For cystic renal masses, diagnostic yield of needle core biopsies have been low and usually biopsy must not be done on these lesions unless accesseble solid areas are present within the lesion (Bosniak IV cysts).[59,60]

HISTOPATHOLOGICAL FEATURES

Renal cell carcinoma arises from the renal tubular epithelium. RCC is characterised by having unique morphological features and distinct genetic abnormalities.[63,64] The diagnosis of RCC is based on unique histomorphological features. IHC and microRNA techniques are used if histological findings are not concluvise in distinguishing the types of RCC.[63] The Fuhrman grading system is used for nuclear grading of RCC. This four-tiered system considers the nuclear features like size of the nucleus and nucleolus, shape of nucleus and nuclear content for nuclear grading.[65]

WHO CLASSIFICATION OF RENAL CELL TUMOURS

- Clear cell Renal cell carcinoma
- Multilocular clear cell Renal cell carcinoma
- Papillary Renal cell carcinoma
- Chromophobe Renal cell carcinoma
- Carcinoma of the collecting ducts of Bellini
- Renal medullary carcinoma
- Xp11 translocation carcinomas
- Carcinoma associated with neuroblastoma
- Mucinous tubular and spindle cell carcinoma
- RCC, unclassified
- Papillary adenoma
- Oncocytoma

CLEAR CELL RCC

This constitutes 70–80% of RCCs. The genetic abnormalities most frequently encountered in this type of RCC are von Hippel-Lindau (VHL) gene mutations , the chromosome 5q duplication and chromosomal deletions at locations of 8p, 14q, 3p, 9p and 16q.[63,66]

Macroscopy

Clear cell renal cell carcinomas are randomly distributed cortical tumours. They are usually solitary and occur with equal frequency in either kidney. Less than 5 percent of cases are multicentric and bilateral.[67] Hereditary cancer syndromes like Von Hippel-Lindau disease are usually characterised by early age of onset, multicentricity and bilaterality.

Clear cell RCCs are globular tumours .They protrude from the renal cortex as a bosselated, rounded mass. The tumour and adjacent kidney interface is usually well demarcated. The tumor is pseudocapsuled with a "pushing margin" . The tumor average size is 7 cm in diameter. In countries where radiologic imaging techniques are widely applied, detection of small tumor lesions is increasing. Size itself is not a determinant of malignancy though increasing size is associated with a higher frequency of metastases. All tumours of the kidney with clear cell type are considered as malignant tumours. Due to the rich lipid content of cells, neutral lipids, cholesterol and phospholipids, the clear cell renal cell carcinoma is typically golden yellow . Cysts, calcification, necrosis and haemorrhage are commonly present. Radiologically10to 15percent of tumours show calcification and ossification within necrotic zones.[68,69]

Microscopy

On hemotoxylin and eosin (H&E) staining under light microscopy, cytoplasm of Clear cell RCC appears more or less empty. This effect is due to the intense glycogen and phospholipid accumulation in the cytoplasm which in turn is attributed to the increase in glucose-6-phosphate levels induced by decreased gluconeogenesis and increased glycolysis.[70,71] In well differentiated tumours, the tumour cell nuclei are more condensed. The tumour cell nuclei exhibit more polymorphism and prominent nucleoli in less differentiated tumours.[72] Eosinophilic or granular appearance of the cytoplasm is an another morphological variant of Clear cell RCC, which in turn is caused by the mitochondrial augmentation.

These tumors are characterised by variable architecture with acinar or tubular patterns. The stroma is poorly defined inspite of rich vasculature surrounding them. Occasionally, scattered bizarre nuclear forms are seen in otherwise typical tumors, a phenomenon similar to that more commonly seen in endocrine neoplasms and which should not be equated to sarcomatoid or anaplastic transformation.[73] The stroma of renal cell carcinoma is nondescript and, in general, not as abundant as in collecting duct carcinoma or transitional cell carcinoma. A lymphocytic infiltrate (mainly composed of T cells) of variable degree is present.

Cases have also been described in which the red blood cells in the stroma form clusters, resulting in a myospherulosis-like appearance.[74]

Immunoprofile

Clear cell RCCs frequently react with antibodies to brush border antigens, low molecular weight cytokeratins(LMWCK), CK19, CK18, AE1, CK8, vimentin and Cam 5.2. Detection of high molecular weight cytokeratins(HMWCK) are rare. The most of clear cell RCCs react positively for renal cell carcinoma marker epithelial membrane antigen and CD10. MUCI and MUC3 are consistently expressed .[75,76]

MULTILOCULAR CYSTIC RCC

This tumor is characterised by numerous cysts in entirity. Within the septa of the cyst lies the small clear cell groups which is similar to clear cell carcinoma - grade I. There is male predominance. Mean age is 51 years.[77]

Multilocular cystic renal cell carcinoma are well-circumscribed with serous or haemorrhagic fluid filled small and large cysts. A fibrous capsule is seen separating this lesion from the normal kidney. More than 20% of tumors have calcification in the septa .Usually a single epithelial cell layer lines the cysts or cyst may lack lining epithelium. The lining cells of the cyst may be plump or flat with a clear to pale cytoplasm.

Occasionally, the lining may be of cells of several layers or a few small papillae are seen.[78] The nuclei are small and spherical with a dense chromatin. Fibrous tissue forming the septa is often densely collagenous. Within some of the septa, epithelial cell collections with a clear cytoplasm are seen. These epithelial cells usually resemble cells those lining the cysts and have small dark nuclei. These epithelial cells resemble histiocytes, or lymphocytes surrounded by retraction artefacts. The cells are strongly positive for cytokeratins(CK) and epithelial membrane antigen(EMA) .

PAPILLARY RCC

Papillary RCC represents about 15% of all renal cell carcinomas. They arise in patients on chronic hemodialysis.[79] Some of the papillary renal cell carcinoma are hereditary, and these have been found to be associated with the c-MET mutation.[80] It has a tendency towards multicentricity and bilaterality. This tumour has a distinct papillary growth pattern, with a solid pattern in undifferentiated areas. The papillary structure are lined by a single layer of neoplastic cells with a fibrovascular core containing foci of lipid-rich macrophages.[81,82]

This tumour can be divided into two types: type 1 papillary RCC, in which the papillae are lined by a single layer of cells; The cells have a pale scanty cytoplasm. Type 2 papillary RCC, in which the papillae are

lined by a pseudostratified epithelium. These cells are characterised by an abundant acidophilic cytoplasm.[83,84] Type 1 tumors that are accompanied by foamy macrophages and psammoma bodies and are immunoreactive for keratin 7 and MUC1.[85,86] When compared to conventional renal cell carcinoma, this tumour has a better prognosis.[87,81]

Papillary RCCs are characterised by the loss of Y chromosomes in males and trisomy of chromosomes 8p, 3q, 7, 16, 12, 20 and17.[63] Papillary renal cell carcinoma can undergo anaplastic or sarcomatoid changes.[88] The presence of numerous foamy macrophages and extensive tumour necrosis has been associated with a more favourable prognosis.[81,89]

CHROMOPHOBE RCC

It comprises approximately 5% of renal epithelial tumours. It has a lobulated surface with one or more solid tumour nodules. The cut surface of this tumour appears homogeneously orange; after formalin fixation, it turns beige or sandy.[90]

Microscopically, the characteristic feature is nesting ('alveolar') arrangement of tumor cells. Microcystic and adenomatous patterns of growth can also be seen sometimes.[91] The tumor cells have sharply defined borders. Cytoplasm is abundant and has a pale, acidophilic

quality. There is often a clear perinuclear region.[92] Pale cytoplasm is due to the presence of numerous cytoplasmic vesicles.[93] With Hale colloidal iron technique, the microvesicles are stained blue.[94] Calcification is seen in nearly half of cases. Immunohistochemically, chromophobe renal cell carcinoma is positive for EMA, ck7, CD9, CD82, paxillin, claudin-7 and -8, Ep-Cam (an epithelial adhesion molecule).[95]

COLLECTING DUCT CARCINOMA

It accounts for approximately less than 1% of RCCs.[66] These tumors are more common in young males. They are centered in the medulla and have a tubulopapillary architecture, and are surrounded by a desmoplastic reaction.[96] The cells have a hobnail pattern with a eosinophilic cytoplasm. The cells usually display (Fuhrman 3 and 4) nuclear features. Both intraluminal and intracytoplasmic mucin may be seen. Atypical changes in the adjacent ducts are common. Cases with signet ring features are also reported.[97]

Vinculin is the immunohistochemical marker for this tumor type. The characteristic feature is a positive reaction to Ulexeuropaeus and coexpression of low molecular weight CKs and high molecular weight CKs. Leu M1 and epithelial membrane antigen has a variable expression.[90] This clinically aggressive tumour, often shows metastases at presentation and characterised by rapid progression.[98] The typical

collecting duct carcinomas has poor prognosis . Two thirds of patients die within two years of diagnosis.

RENAL MEDULLARY CARCINOMA

This is a very rare tumour. This tumour characteristically occurs in young black patients suffering from sickle cell disease.[99] They are centered in medulla and poorly circumscribed . Tumor` mean size is approximately 7 cm. Most of these tumours have multiple areas of haemorrhage and necrosis. Microscopically it exhibits a yolk sac-like , reticular or adenoid cystic appearance and poorly differentiated areas. This tumour has desmoplastic stroma with neutrophils and marginated by lymphocytes.[100] Immunohistochemically, they are consistently positive for CEA. They are often reactive to CK20, CAM5.2, CK7 AE1/AE3, and vimentin. [101] It has a very aggressive behaviour and usually present with metastasis.

RENAL CARCINOMAS ASSOCIATED WITH XP11.2 TRANSLOCATIONS / TFE3 GENE FUSION

These malignancies are characterised by different translocations in chromosome Xp11.2. All of these translocations in turn can cause gene fusions in the transcription factor binding to IGHM enhancer 3 (TFE3) gene. Children and young adults are predominantly affected by this

tumor.[102] These malignancies are usually characterised by an advanced stage of presentation .

On gross examination, they resemble Clear cell RCC and most commonly tan or yellow and often with necrosis and haemorrhage. The most characteristic histopathologic feature is the papillary architecture comprised of cells having a clear to granular eosinophilic type of cytoplasm with distinct cell borders. These cells have vesicular nucleus with prominent nucleoli. In all these tumours, there is constant presence of psammoma bodies.[90]

TFE3 protein has a nuclear immunoreactivity and it is the most chararcteristic immunohistochemical feature of these tumours. 50% of tumors only express cytokeratin and EMA.[102] The tumours are also positive for Renal Cell Carcinoma Marker antigen and CD10.

RENAL CELL CARCINOMA ASSOCIATED WITH NEUROBLASTOMA

It occurs in adolescents with history of childhood neuroblastoma. Subsequent development of renal cell carcinoma in these patient is found to be caused by Neuroblastoma treatment. Median age at the time of diagnosis of Renal cell carcinoma was 13.5 years. Males and females have equal incidence rates.

In these morphologically heterogeneous tumours, some are characterized by solid and papillary architecture. The cells are with abundant eosinophilic cytoplasm and some are with reticular cytoplasm, exhibiting mild to moderate pleomorphism.[103] In other group, the tumours are small, and clear cell RCC were detected incidentally. These tumours are usually positive for vimentin, EMA, and keratins 8, 18, and 20 .They are negative for keratins 7, 14, and 19.

MUCINOUS TUBULAR AND SPINDLE CELL CARCINOMA

For the first time, this entity was included in the current WHO classification . Mean age is 53 year at the time of diagnosis .There is a female predominance. On ultrasound, it is usually found as an incidental mass. They are well circumscribed, grey or light tan with uniform cut surfaces. They are low-grade malignancies.

These tumors were composed of tubules which are tightly packed with pale mucinous stroma separating these tubules. The tubular arrays often have a spindle cell configuration. Distal nephron is likely to be the site of origin. But some believe it to be of proximal tubule origin as a papillary RCC variant. Rare cases exhibit sarcomatoid changes.[90]

RENAL CELL CARCINOMA, UNCLASSIFIED

This group accounts to 4-5% of RCC cases. Renal carcinoma that could not be fit into one of the other categories should be classified into this diagnostic category.[104] Since this variety is comprised of tumours with varying appearances and genetic heterogenecity, it cannot be described to have specific histological features. The features for defining unclassified RCC include

a) Admixture of recognised types,
b) Mucin production,
c) Absence of recognisable epithelial elements with presence of distinct sarcomatoid morphology,
d) presence of both epithelial and stromal elements rarely,
e) cell types with unrecognisable features.

At presentation, in comparison with clear cell RCC, unclassified type was found to have larger size of tumours, increased incidence of adrenal gland invasion, adjacent organs invasion, regional and nonregional lymph nodal involvement and metastasis to bone. On multivariate analysis, Unclassified histology itself was an independent marker for poor outcome. Median survival of patients suffering from unclassified RCC was around 4.3 month.[90]

NUCLEAR GRADING :

Skinner et al first proposed the nuclear grading system. Nuclear morphology was the basis for this grading system. In RCC, for demonstrating this system`prognostic value, Skinner et al carried out a study comprised of 272 patients. This study demostrated a high correlation of nuclear grade with patient survival rate in RCC.[105]

Skinner grading system[106]

- G1 – Nuclei are small, indistinguishable from those seen in normal tubular cells
- G2 – Nuclei are slightly irregular and frequently pyknotic without abnormal nucleoli
- G3 – Nuclei are irregular, enlarged and pleomorphic with prominent nucleoli
- G4 – Nuclei are extremely giant and bizarre

In 1982, the nuclear grading system proposed by Skinner et al was simplified by Furhman et al. This four-tier system used the features such as size of nucleus and nucleoli, shape of nucleus and contents of nuclei. In this system for nuclear grading, regardless of their percentage, highest grade of any of its components is used to classify the entire neoplasm. Worldwide, this grading system is currently used for nuclear grading of RCC.[105]

Fuhrman grading system[107]

- G1 – Nuclei are small, round and uniform (10 μm), with inconspicuous or absent nucleoli.
- G2 – Nuclei are slightly irregular (15 μm), with small nucleoli.
- G3 – Nuclei are very irregular (20 μm), with large and prominent nucleoli.
- G4 – Nuclei exhibit large and pleomorphic often poly-lobed and bizarre (> 20 μm).

This grading system is used to assess the RCC prognosis, especially for conventional and papillary RCC. It is widely acceptable for its simplicity. Its correlation with different pathologic variables has been proven. Most of the controlled studies has confirmed its prognostic value in RCC patients. Poor prognostic outcome has been associated with grade 3 or 4 of this system. Good prognostic outcome has been associated with grade 1 or 2 of this system. Intraobserver variability and interobserver variability has been the problem with this grading system and hence the reproducibility problem among pathologists.[65]

TREATMENT OF LOCALIZED RCC

For localized RCC, radical nephrectomy has been the gold standard treatment option historically.[108] Nowadays, partial nephrectomy

(nephron-sparing surgery - NSS) is the standard treatment option recommended for localised renal neoplasms measuring up to 7 cm in diameter, and for larger neoplasms also, it can be the treatment option, whenever surgically feasible.[109,110] In prospective randomized studies the oncological efficacy of NSS is confirmed, and it has been proved that with NSS, incidence of renal insufficiency and ill effects on day to day health has been reduced, and cardiovascular ill effects also reduced on comparison with radical nephrectomy.[111]

For some of the selected patients with RCC, laparoscopic resection of renal masses has become the one of the standard treatment option. When compared to open surgery, laparoscopic renal surgery is associated with lower rate of morbidity,[110] for localised renal tumours that are not suitable for NSS , laparoscopic nephrectomy is the standard procedure and it also provides an equivalent prognostic outcomes in comparison with open surgery .[112] With experienced hands and with careful selection of patients, laparoscopic partial nephrectomy has been an effective alternative method to partial nephrectomy by open laparotomy.

Partial nephrectomy by means of Robotic-assistance is under trial. Role of lymphadenectomy in the management of RCC patients is currently restricted only for the purpose of staging, principally at the renal hilar region. In patients with retroperitoneal lymph nodal spread,

extended lymphadenectomy may improve the survival rates of RCC patients. In case of patients with preoperative imaging showing normal adrenal gland, routine adrenalectomy is to be done only for large upper pole renal tumors or for renal tumours measuring more than 7 cm diameter.[110]

Minimally-invasive procedures such as

- cryoablation
- percutaneous ablation by means of radiofrequency
- microwave ablation
- high-intensity focused ultrasound (HIFU), and
- laser ablation[113]

are alternative procedure for surgical resection in some of the selective RCC patients such as multiple tumours or poor overall health status. Active follow up can be advised for small renal tumours and treatment can be considered only if it shows significant progression.[110]

TREATMENT OF METASTASIZED RENAL CELL CARCINOMA

The typical feature of RCC is resistance against cytotoxic drugs, radiotherapy, and hormones.[114] Immunotherapy with interleukin-2 (IL-2) or interferon alpha (IFN-α) can produce durable and complete responses. The response rate for IFN-α is found to be 6–15% and for IL-

2, response rate is 7-27%; this provides only a modest benefit in terms of survival in patients with advanced stage of RCC. Currently, adjuvant use with bevacizumab is the only role of immunotherapy in patients with advanced stage of RCC.[110,114]

The Molecular targeted therapies are designed in order to block the critical signalling pathways which underlies the pathogenesis of RCC. Molecular targeted therapies are divided into three categories: multikinase and tyrosine kinase inhibitors, VEGF antibodies and mTOR inhibitors.[115] For metastasized RCC, these targeted therapies are applied by a systemic route.[110] Clinical trials have proven the efficacy of molecular targeted therapies where they have proved to improve both progression-free and overall survival. Since these drugs do not eradicate the disease. Durable remissions can occur.[114,115] Better efficacy, tolerability, and oral administration are the advantages of molecular targeted therapies over immunotherapy.[115]

Patient survival can be improved by means of cytoreductive nephrectomy along with surgical resection of RCC metastases. They have been advised for RCC patients with a good overall health status.[110] Role of palliative surgery for symptomatic brain and bone metastases must be re-evaluated by means of elaborative clinical trials, in view of the recent achievements in molecular targeted therapies;[116] Palliative radiotherapy

is indicated if systemic treatment proves to be of no use. Severe pain due to metastases can be relieved by means of embolization of paravertebral and bone metastases.[110]

PROGNOSTIC AND SURVIVAL FACTORS IN RCC

RCC has a variable clinical course. Incidentally discovered and small tumours have an indolent course even without treatment. Survival rates are poor in metastasized RCC or recurrent disease . The overall RCC prognosis has been greatly improved by means of diagnosis in early stages of the tumor and by means of significant advancement in anatomical imaging, surgical staging and different modes of treatment(both medical and surgical means).[117,5] Stage and grade are currently the most important RCC prognostic factors.[9]

Clinical prognostic factors

Patients who are presenting with clinical symptoms are found to have decreased survival, whereas patients with incidentally found tumors are likely to have a more favourable prognosis, which can be explained by the incidence of smaller size of the renal mass and lower tumour stage at the time of diagnosis.[118,119,120] More than 10% body weight loss in 6 months is found to have significantly lowered survival rate. For predicting poor prognosis, cachexia have been an independent indicator.[64,120] The overall health status, as assessed by the Karnofsky

scales or Eastern Cooperative Oncology Group (ECOG), can estimate the impact of RCC on the overall wellbeing of the patient, and have been accepted as the significant and established prognostic factors for RCC.[64]

Younger age of diagnosis has been established as an independent indicator of a more favourable outcome.[121] Gender does not have any prognostic potential.[119]

Patient survival rate can be correlated with many laboratory indices. Excessive interleukin-6 (IL-6) production by the advanced RCC leads to a relatively high CRP level compared to early stage RCC. This IL-6 is a cytokine with multifunctional growth factor activities and in turn can be a predictor for a more poor prognosis. Poor outcome of the RCC patient can also be predicted by the elevated erythocyte sedimentation rate and thrombocytosis.[64] In addition, haemoglobin, serum calcium, lactate dehydrogenase, albumin, neurone-specific enolase (NSE, γ-enolase) and alkaline phosphatase are also found to be of prognostic value to some extent in RCC.[122]

Prognostic anatomical factors: stage at diagnosis

The tumor staging is currently the most reliable indicator in RCC prognosis. For localized stage of disease, 5-year survival rates following radical nephrectomy is generally 75–95%; for locally advanced stage of

RCC, it is 65–80%; for tumours extending into inferior vena cava, it is 40–60%; for RCC with lymph nodal extension, it is 10–20%; and for RCC with metastasis, it is 0–5%.[64] Staging takes into account features like size of the tumour, venous extension , invasion into the renal capsule, involvement of adrenal gland, lymph nodal involvement and metastasis to distant organs, and all of which are established independent prognostic markers and when they are assessed in combination by means of staging, provide the most dependable prognostic information in RCC patients.[9,123] The TNM classification for staging of RCC was revised recently in 2009.[124] In comparison to the previous 2002 version of staging[125], T2 tumour class is now sub classified into T2a(tumours more than 7 cm but less than 10 cm in diameter) and T2b(tumours more than 10 cm in diameter), but both not extending beyond the limits of the kidney. In addition, RCC with a tumour thrombus extending only into the corresponding renal vein is now staged as T3a and invasion into adjacent adrenal gland is now staged as T4.[110]

Histological prognostic factors

Despite strong criticism regarding the predictive value and validity of the Fuhrman grading system, untill now it is considered the most reliable histological grading system available for RCC . It is accepted one

of the independent prognostic marker for clear cell variety of RCCs.[121] The RCC-specific 5-year survival rate is 89% in case of grade 1 tumours, 65% in case of grade 2 tumours and 46% in case of grades III-IV tumours.[16] In general, the overall prognosis for papillary type of RCC and chromophobe type of RCC is much better than overall prognosis of clear cell type of RCC. The overall survival rate of collecting duct type of RCC is poor.[9,110] Among papillary type of RCCs, type 1 tumours generally have a better prognosis than the prognosis of type 2, which itself is one of the independent indicators of a poor prognosis.[84] Among the different histological features of RCC, microscopic invasion of veins(MVI), sarcomatoid differentiation, collection system invasion and areas of tumoural necrosis are correlated with decreased survival rates. Cystic component of RCC is accepted as an independent marker to indicate more benign clinical outcome.[110,121]

Molecular prognostic factors

Numerous molecular markers are being investigated which includes carbonic anhydrase IX (CaIX), vascular endothelial growth factor (VEGF), hypoxia-inducible factor (HIF), Ki67 (proliferation), p53, E-cadherin, C-reactive protein (CRP), CD44 (cell adhesion) and osteopontin. Gene expression profiling seems a promising method, to identify new relevant prognostic factors .[126]

Prognostic Factors In Metastasized Renal Cell Carcinoma

In advanced RCC, classic anatomical and histological features of the primary tumour had limited predictive value.[9] The prognostic factors identified in metastasized disease are performance status, time of appearance of metastases, number and locations of metastatic sites, prior nephrectomy and surgical resection of metastases.[120] Metastases to bone have been regarded as a marker of shorter survival. Number of metastatic sites is considered as a more important prognostic marker than location of metastasis.[64]

IMMUNOHISTOCHEMISTRY:

Albert Coons et al in 1941 first labelled antibodies directly with fluorescent isocyanate. Nakane and Pierce et al in 1966, introduced the indirect labeling technique in which the unlabelled antibody is followed by second antibody or substrate. Various stages of development of Immunohistochemistry include peroxidase – antiperoxidase method (1970), alkaline phosphatase labeling (1971), avidin biotin method (1977) and two layer dextrin polymer technique (1993).[127]

ANTIGEN RETRIEVAL:

Antigen retrieval can be done by the following different techniques to unmask the antigenic determinants of fixed tissue sections.

1. Proteolytic enzyme digestion
2. Microwave antigen retrieval
3. Pressure cooker antigen retrieval
4. Microwave and trypsin antigen retrieval

PROTEOLYTIC ENZYME DIGESTION:

Huank et al in 1976 introduced this technique to breakdown formalin cross linkages and to unmask the antigen determinants. The most commonly used enzymes include trypsin and proteinase.[128] The disadvantages include over digestion, under digestion and antigen destruction.

MICROWAVE ANTIGEN RETRIEVAL:

This is a new technique most commonly used in current practice. Microwave oven heating involves boiling formalin fixed paraffin sections in various buffers for rapid and uniform heating.[127]

PRESSURE COOKER ANTIGEN RETRIEVAL:

Miller et al in 1995 compared and proved that pressure cooking method has fewer inconsistencies, less time consuming and can be used to retrieve large number of slides than in microwave method.[129]

PITFALLS OF HEAT PRETREATMENT:

Drying of sections at any stage after heat pretreatment destroys antigenicity. Nuclear details are damaged in poorly fixed tissues. Fibers and fatty tissues tend to detach from slides while heating. Not all antigens are retrieved by heat pretreatment and also some antigens like PGP 9.5 show altered staining pattern.

DETECTION SYSTEMS:

After addition of specific antibodies to the antigens, next step is to visualize the antigen antibody reaction complex. The methods employed are direct and indirect methods.

In the direct method, primary antibody is directly conjugated with the label. Most commonly used labels are flouro-chrome, horse radish peroxidase and alkaline phosphatase. Indirect method is a two-step method in which labelled secondary antibody reacts with primary antibody bound to specific antigen. The use of peroxidase enzyme complex oravidin biotin complex further increases the sensitivity of immunohistochemical stains.[127]

In 1993, Pluzek et al introduced enhanced polymer one step staining, in which large numbers of primary antibody and peroxidase

enzymes are attached to dextran polymer back bone. This is the rapid and sensitive method.[130]

Dextran polymer conjugate two step visualization system is based on dextran technology in Epos system. This method has greater sensitivity and is less time consuming.

Ki-67

Ki-67 also recognized as MKI67 is a protein encoded by the MKI67 gene [131] which was discovered by Gerdes. Originally this protein was defined by the prototype monoclonal antibody Ki-67 and it was generated by immunizing mice with nuclei of the Hodgkin lymphoma cell line L428. It was named based on the city of origin (Kiel, Germany) and the number of the original clone in the 96-well plate.

Ki-67 is a nuclear protein that is necessary for cellular proliferation and ribosomal RNA transcription. It is present during all active phases of the cell cycle (G1, S, G2, and M), but is absent from resting cells (G0). The protein is predominantly localized in the peri-nucleolar region in the G 1 phase, in the later phases it is also detected throughout the nuclear interior, being predominantly localized in the nuclear matrix having a half life of is 60-90 minutes. In mitosis, it is present on all chromosomes.[131] Thus Ki-67 is an excellent proliferation marker to determine the growth

fraction of a specified cell population and is widely as a proliferation marker in many of the human tumours. The fraction of Ki-67-positive tumor cells is often associated with the clinical course of various neoplasms. The monoclonal antibody generally used to detect the Ki-67 antigen is MIB-1. One of its major merits over the original Ki-67 antibody is that it can be applied on formalin-fixed paraffin-embedded sections, after heat-mediated antigen retrieval. Ki-67 labeling index is calculated by the percentage of tumours cells showing distinct brown staining of the nucleus with strong intratumoural heterogeneity.

Studies on RCC, gastric cancer , bladder cancer , lymphomas, colorectal cancer and breast cancer have shown that overexpression of Ki67 is correlated with biological behaviour and prognosis of these malignancies.[132]

The other methods of detection of Ki-67 are by Western blot analysis and immunofluorescence. The various other markers of proliferation include AgNOR staining, PCNA and Topoisomerase II. The novel markers being evaluated for identifying cell proliferation include Fen-1, MCM proteins (mini-chromosome maintenance), mitosin, polo – like kinase and claspin.

P53

p53 was first identified in 1979 by David P. Lane, Lionel, Lloyd Old, and Crawford Arnold Levine. in 1985, human TP53 gene was cloned. The role of P53 as a tumor suppressor gene was discovered by Bert Vogelstein in 1989. It is considered as the "Guardian of the genome". This tumour suppressor gene is located on chromosome 17p13.1. It encodes a nuclear phosphoprotein of 53kDa.[133] p53 plays a central role in cell – cycle regulation, in cell apoptosis and in DNA repair. When there is cellular insult or DNA damage there is increased p53 production; then it induces cell cycle arrest at the G1/S junction. Therefore, for control of tumor growth, apoptosis and maintaining genome stability, p53 is essential. Normal p53 protein, is rapidly removed from the nucleus but mutant forms have prolonged half-life.This favours intranuclear accumulation and so it can be detectable immuno-histochemically. P53 appears mutated in a wide variety of human carcinomas, such oral and oropharyngealcarcinoma, colorectal carcinoma, breast carcinoma, esophageal carcinoma, gall bladder carcinoma and gastric carcinoma. In numerous studies there was correlation between over expression of p53 gene and the poor prognosis in patients with these tumors. The p53 is also involved in regulating the metastasis-associated genes. These genes are integrin, Maspin, matrix

metallo-proteinase-2 (MMP-2), MMP-13 and the tissue inhibitor of metalloproteinase-3 (TIMP3).

P53 appears mutated in about 50% of many malignancies, but in RCC, incidence of p53 mutations is low. P53 mutations has been detectedin 3-33% of patients with RCC. Although in the majority of RCCs p53 remains wild type, this does not mean, that it is functional. P53 function can also be repressed by mechanisms, which involve loss of positive regulators, such as Arf or overexpression of natural negative regulators,MDM2 or MDMX or by viral proteins, such as E6 of the human papilloma virus.

On comparing the association of p53 expression and nuclear grade, there are number of controversial studies. Some investigators have found no association but some of them demonstrated a strong relationship. However, p53 is considered as a potential marker in determining prognosis of patients with RCC.[133] It is now known that like melanoma, RCC also belongs to the type of tumors with a low incidence of p53 mutations when compared to prostate and bladder.[134]

The most commonly used methods for detection of p53 mutations includes immunohistochemistry, polymerase chain reaction-single-strand conformation polymorphism(PCR – SSCP), flow-cytometry and genomic

sequencing. Although sequencing is the most unambiguous method, it is technically cumbersome. Therefore, both immune-detection and PCR have been used as alternative methods.

MUC1

Mucins are high-molecular-weight glycoproteins with 4200 kDa with oligosaccharides are attached to an apomucin protein by O-glycosidic linkage.[135]

The MUC1 gene is located on chromosome 1q21-24 .It is a member of mucin family which encodes a transmembrane glycoprotein. MUC1 is membrane-associated and membrane-secreted. MUC1 also is known as polymorphic urinary mucin, or PUM, and epithelial membrane antigen (EMA). MUC1 has a apical membranous distribution of many glandular epithelia like epithelium of the colon, breast, lung, pancreas and kidney. MUC1 is supposed to play a role in cellular polarity, cell adhesion and signal transduction. In the kidney, MUC1 is expressed in normal distal convoluted tubules, collecting ducts .[8]

Sialylated MUC1 mucin expressed on tumor cells suppresses cellmatrix adhesion and homotypic cellular aggregation and promotes invasion. In vitro, sialylated MUC1 mucin also inhibits cytotoxic

lymphocyte–target cell interactions. They also induce apoptosis of lymphocytes.

Thus, with these findings cancer cells with a high level of sialylated MUC1 expression are able to detach easily from the primary lesion and they survive in circulation and in distant organs of metastasis by escaping immune surveillance.[136]

In many epithelial cancers, there is loss of polarized cellular expression and there is diffuse circumferential distribution. These variations of expression of MUC1 in malignant cells are suspected to be responsible for metastatic dissemination by destabilization of cell-cell and cell–extracellular matrix interactions. In various studies, MUC1 is considered as a marker of tumor progression and prognosis.[8]

MATERIALS AND METHODS

MATERIALS AND METHODS

This study is a combined retrospective and prospective study of renal cell carcinoma, conducted in the Institute of Pathology, and Rajiv Gandhi Government General Hospital, Chennai for a period of 3 years between august 2011 to august 2014 .

Total of 52 cases of resected specimens of renal cell carcinoma were received for histopathological examination in Madras Medical College during the period between august 2011 to august 2014.

INCLUSION CRITERIA:

All resected specimens of renal cell carcinoma, irrespective of the age and stage were included for the study.

EXCLUSION CRITERIA:

1. Renal biopsy specimen
2. Renal malignancies other than renal cell carcinoma
3. Nephrectomy done for Benign and non-neoplastic lesion of kidney

METHOD OF DATA COLLECTION:

Detailed history of the cases regarding age, sex, clinical presentation, investigations done along with the findings, type of procedure done were obtained for all the renal cell carcinoma specimens

received during the period of study. Haematoxylin and Eosin stained 4 micron thick sections of the paraffin tissue blocks of the cases were prepared from nephrectomy specimens and cases reported as renal cell carcinoma were selected. 40 patients were randomly selected for Immunohistochemical analysis using ki67, p53 and MUC1

Variables studied:

The following clinical and pathological parameters were evaluated:

Age, gender, size, laterality (right or left side), histological types (clear cell RCC, papillary RCC, chromophobe RCC, unclassified RCC)

Nuclear grading according to FURHMAN grading system.[107]

- G1 – Nuclei are small, round and uniform (10 μm), with inconspicuous or absent nucleoli.
- G2 – Nuclei are slightly irregular (15 μm), with small nucleoli.
- G3 – Nuclei are very irregular (20 μm), with large and prominent nucleoli.
- G4 – Nuclei exhibit large and pleomorphic often poly-lobed and bizarre (> 20 μm).

Presence of capsular infiltration, renal vessel invasion, ureter invasion and lymph node involvement, distant metastasis and TNM STAGING (ANNEXURE 3)were performed. Representative formalin

fixed, paraffin embedded tissue samples were subjected to immunohistochemical analysis with a panel of 3 markers i.e., ki67, p53 and MUC1

Antigen	Vendor	Species (clone)	Dilution	control
KI67	PATHINSITU	MOUSE	Ready to use	Malignant phyllodes
P53	DAKO	MOUSE	Ready to use	Colonic malignancy
MUC1	PATHINSITU	RABBIT	Ready to use	Distal Convoluted Tubule

Immunohistochemistry procedure:

Slide Preparation:

1. Sections with a thickness of 4 µ were cut from formalin fixed paraffin embedded tissue samples and transferred to gelatin-chrome alum coated slides.
2. The slides were incubated for overnight at 58ºC.
3. The sections were deparaffinised in xylene for 15 minutes x 2 changes.

4. The sections were dehydrated with absolute alcohol for 5 minutes for 2 changes.
5. The sections were then washed in tap water for 10 minutes.
6. The slides were then immersed in distilled water for 5 minutes.

Antigen Retrieval:

1. Heat induced antigen retrieval was done with microwave oven in appropriate temperature with appropriate buffer for 20 minutes. This step unmasks the antigenic determinants of fixed tissue sections.
2. The slides were then cooled to room temperature for 20 minutes and washed in running tap water for 5 minutes.
3. The slides were then rinsed in distilled water for 5 minutes.
4. They were washed with appropriate wash buffer (phosphate buffer) for 5 minutes x 2 changes.
5. Peroxidase block was applied over the sections for 10 minutes.
6. The slides were washed in phosphate buffer for 5 minutes x 2 changes.
7. Sections were covered with protein block for 5 minutes.

Antibody application:

1. The sections were drained (without washing) and appropriate primary antibody was applied over the sections and incubated for 30 minutes.
2. The slides were washed in phosphate buffer for 5 minutes x 2 changes.
3. The slides were covered with Primary antibody amplifier for 10 minutes.
4. The slides were washed in phosphate buffer for 5 minutes x 2 changes.
5. The slides were covered with HRP micropolymerQuanto for 10 minutes.
6. The slides were washed in phosphate buffer for 5 minutes x 2 changes.

Chromogen application:

1. DAB substrate was prepared by diluting 1 drop of DAB Quantochromogen to 1 ml of DAB Quanto buffer.
2. DAB substrate solution was applied on the sections for 5 minutes.
3. The slides were washed in distilled water for 2 minutes.
4. The sections were counterstained with Hematoxylin for 2 seconds.
5. The slides were washed in running tap water for 5 minutes.

6. The slides were air dried, cleared with xylene and mounted with DPX.

INTERPRETATION AND SCORING SYSTEM

Ki67

The immunohistochemically stained slides were analyzed for the presence of reaction and percentage of cells stained. Immunoreactivity was identified by nuclear brown color. The percentage of nuclei with immunoreactive ki67 was counted for each tumor slide. Immunoreactivity was classified as continuous data from undetectable levels (0%) to homogeneous (100%). The reaction is considered positive when 10% or more of the tumor cells showed staining, according to previous study.[134]

P53

Immunoreactivity was identified by nuclear brown color. The percentage of nuclei with immunoreactive p53 was counted for each tumor slide. Immunoreactivity was classified as continuous data from undetectable levels (0%) to homogeneous (100%).

Expression of p53 was evaluated separately using the following scale:[133]

- 3+ = high level (91-100% of positive cells)
- 2+ = medium level (11-90% of positive cells)

- 1+ = low level (up to 10% of positive cells)
- 0= negative cells (0% of positive cells).

For purpose of statistical analysis a sample is said to be positive if 5% of cells are positive for p53.[137]

MUC1

A cell was estimated as positive when the cytoplasm, cell membrane, or both were stained. The percentage of positively stained cells (positive rate) was determined for each tumor. Immunoreactivity was categorized as follows:

0 - no reactivity

1 - less than 10% of cancer cells positive

2 - 10–25% positive

3 - 25–50% positive

4 - 50–75% positive

5 - 75–90% positive

6 - more than 90% of cancer cells positive.

For statistical analysis, in accordance with previous studies, sample with more than 10% of tumor cells positive immunostaining were considered as positive .[135]

STATISTICAL ANALYSIS:

The statistical analysis was performed using statistical package for social science software version 15.5 which consisted computing the frequency counts and percentages for qualitative variables and mean for the quantitative variables. The expression of KI67, P53, MUC1 was correlated with clinico-pathological factors like age, gender, tumor size, histological types, nuclear grade, stage using pearson's chi-square test. The p value was considered significant if below 0.05.

OBSERVATION AND RESULTS

OBSERVATION AND RESULTS

From august 2011 to august 2013, a total of 31,237 cases were received for histopathological examination in the institute of pathology, Madras Medical College. Among this, 52 were nephrectomy specimens done for RCC. Of this, 36 were clear cell RCC, 8 were papillary RCC, 2 were Chromophobe RCC, 6 were unclassified RCC. Clear cell RCC was the most common type**.**

TABLE 1: DISTRIBUTION OF TYPE OF RENAL CELL CARCINOMA

TYPE	N (%)
Clear cell RCC	36 (69.23%)
Papillary RCC-Type I	7(13.5%)
Papillary RCC-Type II	1(1.9%)
Chromophobe RCC	2 (3.8%)
RCC, Unclassified	6 (11.5%)
TOTAL	52(100%)

Among the 52 cases of renal cell carcinoma, maximum 36 (69.23 %) cases were of clear cell RCC and 2nd maximum 7 (15.3 %) cases were of papillary RCC. Minimum cases 2(3.8%) were of chromophobe RCC. Unclassified cases were 6(11.5%). (TABLE1&CHART 1)

TABLE 2: AGE WISE DISTRIBUTION OF RENAL CELL CARCINOMA

AGE GROUP	RCC (%)
21 - 30 years	1 (1.92 %)
31 - 40 years	9 (17.31 %)
41 - 50 years	16 (30.77 %)
51 - 60 years	12 (23.08%)
61 - 70 years	11 (21.15%)
>70 years	3(5.77 %)
Total	52 (100%)

Among the 52 cases of renal cell carcinoma, maximum 16 (30.77 %) cases were reported in 41-50 years age group and 2nd maximum 12 (23.08%) cases were reported in 51-60 years age group. Minimum age group reported was 21-30 years. (TABLE 2 & CHART 2)

TABLE 3: AGE WISE DISTRIBUTION IN TYPES OF RENAL CELL CARCINOMA

Age	Clear cell RCC	Papillary RCC	Chromophobe RCC	Unclassified RCC
21-30 Years	1(2.7%)	0(0%)	0(0%)	0(0%)
31-40 years	6(16.6%)	2(25%)	0(0%)	1(16.6%)
41 - 50 years	11(30.5%)	3(37.5%)	0(0%)	2(33.3%)
51 - 60 years	9(25%)	0(0%)	0(0%)	3(50%)
61 - 70 years	7(19.4%)	3(37.5%)	1(50%)	0(0%)
>70 years	2(5.6%)	0(0%)	1(50%)	0(0%)
Total	36(100%)	8(100%)	2(100%)	6(100%)

Among 36 cases of clear cell renal cell carcinoma, maximum 11 (30.5%) cases were reported in 41-50 years age group and the lowest incidence of 1(2.7%) case reported in age group 21-30 years and 2nd lowest incidence of 2 cases were reported in age group >70 years. Totally 8 cases of papillary RCC were reported and of this, 75% cases were reported in the age group of 41-70%; lowest age group 31-40 years

were reported in 25% of papillary RCC cases. Only 2 cases of chromophobe RCC were reported which are more than 60 years. Of the unclassified type 3(50%) cases were reported in the age of 51-60 years.(TABLE 3)

TABLE 4: SEX WISE DISTRIBUTIONS OF RENAL CELL CARCINOMA

SEX	RCC (%)
Male	38(73.08 %)
Female	14 (26.92 %)
Total	52

Among the 52 cases of renal cell carcinoma, 38(73.08 %) cases were reported in males and 14 (26.92 %) cases were reported in females. The male to female ratio was 2.7:1. (TABLE 4& CHART 3)

TABLE 5: SIDE WISE DISTRIBUTION OF RENAL CELL CARCINOMA

SIDE	RCC (%)
Right	35 (67.31 %)
Left	17 (32.69 %)
Total	52

Among the 52 cases of renal cell carcinoma, 35 (67.31 %) cases were reported on right side and 17 (32.69 %) cases were reported on left side. The right to left side ratio was 2.1:1. (TABLE 5 & CHART 4)

TABLE 6: DISTRIBUTION OF RENAL CELL CARCINOMA BASED ON THE SIZE

Size	No Of Cases	Percentage %
≤5 cm	14	26.9
>5 cm	38	73.1
Total	52	100

In this study 38(73.1%) tumors were of size more than 5 cm. 14(26.9%) tumors were of size less than or equal to 5 cm. Median diameter of the tumor is 7.5 cm. (TABLE 6 & CHART 5)

TABLE 7: DISTRIBUTION OF TYPES OF RENAL CELL CARCINOMA BASED ON THE SIZE

Size	Clear Cell RCC (%)	Papillary RCC (%)	Chromophobe RCC (%)	Unclassified RCC (%)
≤5 cm	11(30.6%)	3(37.5%)	0(0%)	0(0%)
>5 cm	25(69.4%)	5(62.5%)	2(100%)	6(100%)
Total	36	8	2	6

In clear cell RCC, 69.4% were more than 5 cm. In papillary RCC 62.5% were more than 5 cm. In chromophobe RCC and unclassified type, all the tumors were more than 5 cm. (TABLE 7& CHART 6)

TABLE 8: GRADE AT PRESENTATIONAMONG RENAL CELL CARCINOMA

GRADE	RCC (%)
1	9(17.3 %)
2	16(30.8 %)
3	17(32.7%)
4	10(19.2 %)
Total	52(100%)

Among the 52 cases of renal cell carcinoma, maximum 17(32.7%) cases were of grade 3 and 2nd maximum 16(30.8 %) cases were of grade 2. (TABLE 8 & CHART 7)

TABLE 9: GRADE AT PRESENTATION AMONG RENAL CELL CARCINOMA

NUCLEAR GRADE	CLEAR CELL RCC	PAPILLARY RCC	CHROMOPHOBE RCC	UNCLASSIFIED RCC
1	8	1	0	0
2	12	3	1	0
3	11	2	1	3
4	5	2	0	3
Total	36	8	2	6

In this study clear cell RCC cases were seen in all grades. Most of them were in grade 2 and 3. Most of the papillary RCC were of grade 2. One of the chromophobe RCC was grade 2 and the other was grade 3.Unclassified RCC were of grade3 and grade 4.(TABLE 9 & CHART 8)

TABLE 10: DISTRIBUTION OF CAPSULE INFILTRATION AMONG RENAL CELL CARCINOMA

Capsule infiltration	RCC (%)
Present	19(36.5%)
Absent	33(%)
Total	52

Among the 52 cases of renal cell carcinoma, capsule infiltration was present among 17(36.5 %) cases and absent among 33(63.5 %) cases. (TABLE 10)

TABLE 11: DISTRIBUTION OF PERINEPHRIC TISSUE INVOLVEMENT AMONG RENAL CELL CARCINOMA

PERINEPHRIC TISSUE INVOLVEMENT	RCC (%)
Present	13(25%)
Absent	39(75%)
Total	52

Among the 52 cases of renal cell carcinoma, perinephric tissue involvement was present among 13(25 %) cases and absent among 39 (75 %) cases. (TABLE 11)

TABLE 12: DISTRIBUTION OF GEROTA'S FASCIA INVOLVEMENT AMONG RENAL CELL CARCINOMA

GEROTA'SFASCIA INVOLVEMENT	RCC (%)
Present	5(9.6%)
Absent	47(90.4%)
Total	52

Among the 52 cases of renal cell carcinoma, Gerota's fascia involvement was present among 5(9.6 %) cases and absent among 47 (90.4 %) cases. (TABLE 12)

TABLE 13: DISTRIBUTION OF URETER INVASION AMONG RENAL CELL CARCINOMA

URETER INVASION	RCC (%)
Present	4(7.7%)
Absent	48(92.3%)
Total	52

Among the 52 cases of renal cell carcinoma, ureteric invasion was present among 4(7.7 %) cases and absent among 48(92.3%) cases. (TABLE 13)

TABLE 14: DISTRIBUTION OF RENAL VESSEL INVASION AMONG RENAL CELL CARCINOMA

RENAL VESSEL INVASION	RCC (%)
Present	10(19.2%)
Absent	42(80.8%)
Total	52

Among the 52 cases of renal cell carcinoma, renal vessel invasion was present among 10(19.2%) cases and absent among 42(80.8 %) cases. (TABLE 14)

TABLE 15 : DISTRIBUTION OF ADRENAL INVOLVEMENT AMONG RENAL CELL CARCINOMA

ADRENAL INVOLVEMENT	RCC (%)
Present	4(7.7%)
Absent	48(92.3%)
Total	52

Among the 52 cases of renal cell carcinoma, adrenal involvement was present among 4(7.7 %) cases and absent among 48(92.3 %) cases. (TABLE 15)

TABLE 16: DISTRIBUTION OF REGIONAL LYMPHNODAL INVOLVEMENT AMONG RENAL CELL CARCINOMA

Regional lymphnodal involvement	RCC (%)
Present	4(7.69 %)
Absent	48(92.31 %)
Total	52

Among the 52 cases of renal cell carcinoma, regional lymphnodal involvement was present among 4(7.69 %) cases and absent among 48(92.31 %) cases.

TABLE 17: DISTRIBUTION OF DISTANT METASTASIS AMONG RENAL CELL CARCINOMA

DISTANT METASTASIS	RCC (%)
Present	3(5.8%)
Absent	49(94.2 %)
Total	52

Among the 52 cases of renal cell carcinoma, distant metastasis was present among 3(5.8 %) cases and absent among 49(94.2 %) cases.

TABLE 18: T stage AT PRESENTATION AMONG RENAL CELL CARCINOMA

T stage at presentation	RCC (%)
T1a	7(13.5%)
T1b	11(21.6%)
T2a	9 (17.3%)
T2b	4(7.7%)
T3a	11(21.6%)
T3b	3(3.8%)
T3c	1(1.9%)
T4	6 (11.5%)
Total	52

Among the 52 cases of renal cell carcinoma, maxium 11(21.6%) cases were presented at T1b stage and T3a. Minimum 6(11.5%) cases were presented at T4 stage. (TABLE 18 & CHART 9)

TABLE 19: T STAGE AT PRESENTATION AMONG TYPES OF RENAL CELL CARCINOMA

T STAGE	CLEAR CELL RCC	PAPILLARY RCC	CHROMOPHOBE RCC	UNCLASSIFIED RCC
T1	12	6	0	0
T2	11	1	1	0
T3	9	1	1	4
T4	4	0	0	2
TOTAL	36	8	2	6

Most of the clear cell RCC and papillary RCC were presented in T1stage. Chromophobe RCC were presented in T2 stage and T3 stage. Most of the unclassified type presented in T3 stage. (TABLE 19)

TABLE 20: STAGE AT PRESENTATION AMONG RENAL CELL CARCINOMA

STAGE	RCC (%)
1	18(34.6%)
2	13(25%)
3	14(26.9%)
4	7(13.5%)
Total	52(100%)

Among the 52 cases of renal cell carcinoma, maximum 18(34.6 %) cases were presented at stage 1 and 2nd maximum 14(26.9 %) cases were presented at stage 3. Minimum cases 7(13.5%) were presented at stage 4. (TABLE 20)

TABLE 21 : STAGE AT PRESENTATION AMONG TYPES OF RENAL CELL CARCINOMA

STAGE	CLEAR CELL RCC	PAPILLARY RCC	CHROMO PHOBE RCC	UNCLASSIFIED RCC	TOTAL
1	12	6	0	0	18
2	11	1	1	0	13
3	9	1	1	3	14
4	4	0	0	3	7
Total	36	8	2	6	52

Most of the clear cell RCC were presented in stage 1. Most of the papillary RCC were presented in stage 1. Chromophobe RCC were presented in stage 2 and stage 3. Unclassified RCC were presented in stage 3and 4. (TABLE 21 & CHART 10)

TABLE 22 :DISTRIBUTION OF CASES ACCORDING TO KI67 EXPRESSION IN RENAL CELL CARCINOMA

KI67	RCC (N)	Percentage
Positive	29	72.5%
Negative	11	27.5%
Total	40	100%

In this study 72.5% of tumors were positive for KI67 and 27.5% were negative for KI67. (TABLE 22 & CHART 11)

TABLE 23 : DISTRIBUTION OF CASES ACCORDING TO KI67 EXPRESSION IN TYPES OF RENAL CELL CARCINOMA

Types of RCC	KI67 Positive	KI67 Negative	Total
Clear cell RCC	20(74%)	7(26%)	27(100%)
Papillary RCC	5(71.4%)	2(28.6%)	7(100%)
Chromophobe RCC	2(100%)	0(0%)	2(100%)
Unclassified RCC	2(50%)	2(50%)	4(100%)
Total	29	11	40
Chi Square Test	P Value-0.612		

In this study, 74% of clear cell RCC were positive for KI67. In papillary RCC, 71.4% were positive for KI67. All chromophobe RCC were positive for KI 67. 50% of unclassified tumors were positive for KI67. There is no significant correlation between KI67 expression and tumor types. (TABLE 23 & CHART 12)

TABLE 24 : DISTRIBUTION OF CASES ACCORDING TO KI67 EXPRESSION IN CORRELATION WITH NUCLEAR GRADE

NUCLEAR GRADE	KI67 positive	KI67 negative	Total
Grade 1	2(28.5%)	5(71.4%)	7(100%)
Grade 2	7(63.6%)	4(36.4%)	11(100%)
Grade 3	11(91.7%)	1(8.3%)	12(100%)
Grade 4	9(90%)	1(10%)	10(100%)
Total	29	11	40
Chi-square test	P value- 0.012		

In this study in grade 1 tumors, 71.4% were negative for KI67. In grade 2 tumors, 63.6% were positive for KI67. In grade 3 tumors, 91.7% were positive for KI67. In grade 4 tumors, 90% were positive for KI67. There was positive correlation between KI67 and nuclear grade. (TABLE 24 & CHART 13)

TABLE 25 : DISTRIBUTION OF CASES ACCORDING TO MEAN KI67 LABELLING INDEX IN CORRELATION WITH NUCLEAR GRADE

Nuclear grade	NO.OF CASES	Mean KI 67 LI
G1	7	5.6%
G2	11	16.7%
G3	12	36.9%
G4	10	59.7%

In this study in grade 1 tumors, mean labelling index(LI) was 5.6%. In grade 2 tumors, mean labelling index was 16.7%. In grade 3 tumors, mean labelling index was 36.9%. In grade 4 tumors, mean labelling index was 59.7%. There is increase in proliferative index with increase in nuclear grade. (TABLE 25 & CHART 14)

TABLE 26: DISTRIBUTION OF CASES ACCORDING TO LOW AND HIGH LEVEL OF KI 67 EXPRESSION IN CORRELATION WITH NUCLEAR GRADE

NUCLEAR GRADE	KI 67 1-10%(Low)	KI 67 >10%(high)
Grade 1	4	2
Grade 2	3	7
Grade 3	1	10
Grade 4	1	9
Total	9	28
Chi-Square Test	P value 0.036	

In grade 1 tumor, 4 (66.6%) showed expression <10%.

In grade 2 tumor, 7(77.7%) showed expression >10%.

In grade 3 tumor, 10(90%) showed expression >10%.

In grade 4 tumor, 1(90%) showed expression >10%. (TABLE 26)

TABLE 27: DISTRIBUTION OF CASES ACCORDING TO LOWAND HIGH LEVEL OF KI 67 EXPRESSION IN TYPES OF RENAL CELL CARCINOMA

Types of RCC	KI 67 1 -10% (Low)	KI 67 >10%(high)	Total
Clear cell RCC	6	19	25
Papillary RCC	1	5	6
Chromophobe RCC	0	2	2
Unclassified RCC	2	2	4
Chi Square Test	P value 0.519		

In this study, 19(76%) of clear cell RCC had KI 67 expression >10%.In papillary RCC, 5(83%) had expression >10%. All chromophobe RCC had expression >10%.(TABLE 27)

CORRELATION OF KI67 EXPRESSION WITH OTHER CLINICO -PATHOLOGICAL FACTORS

TABLE 28: CORRELATION OF AGE WITH KI 67 EXPRESSION IN RCC

AGE	KI67 POSITIVE	KI67 NEGATIVE	TOTAL
<=50	15	7	22
>50	14	4	18
Chi-square test	P value-0.499		

In this study, among 22 cases, 15 were positive for KI67 expression in age less than or equa to 50 years. In the age greater than 50 years, 14 cases were positive for KI 67. There is no correlation between age and KI67 expression . (TABLE 28)

TABLE 29: CORRELATION OF SEX WITH KI67 EXPRESSION IN RCC

SEX	KI67 POSITIVE	KI67 NEGATIVE	TOTAL
Male	21	7	29
Female	8	4	11
Chi-square test	P Value-0.589		

In this study, among 29males, KI67 expression was seen in 21; Among 11 females, KI67 expression was seen in 8. There is no correlation between sex and KI67. (TABLE 29)

TABLE 30: CORRELATION OF SIZE WITH KI67 EXPRESSION IN RCC

SIZE	KI67 POSITIVE	KI67 NEGATIVE	TOTAL
<=5CM	6	4	10
>5 CM	23	7	30
Chi-square test	P VALUE-0.307		

In this study, 10 tumors were of size less than or equal to 5 cm . Of this, 6 were positive for KI67. There is no correlation between size of the tumor and KI67 expression. (TABLE 30)

TABLE 31: CORRELATION OF STAGE WITH KI67 EXPRESSION IN RCC

STAGE	KI67 POSITIVE	KI67 NEGATIVE	TOTAL
1	6	8	14
2	8	1	9
3	11	1	12
4	4	1	5
Chi-square test	P VALUE- 0.021		40

Among 17 stage 3 and stage 4 cases, 15 were positive for positive for KI67. Among 24 stage 1 and stage 2 cases, 14 were positive for KI67. Stage 3 and 4 shows more positivity when compared to stage 1 and 2. There is positive correlation between stage and KI67 expression in this study. (TABLE 31)

TABLE 32 : DISTRIBUTION OF CASES ACCORDING TO P53 GRADING IN RENAL CELL CARCINOMA

P53 GRADE	TOTAL	PERCENTAGE
0	20	50%
1+(1-10%)	10	25%
2+(11-90%)	9	22.5%
3+(91-100%)	1	2.5%
TOTAL	40	100%

In this study, 20(50%) of RCC cases were not immunoreactive for P53. 25% of RCC expressed P53 in the range of 1-10%. Medium expression of P53 was seen in 22.5% of cases. Maximum expression of P53 was seen in 2.5% of cases. (TABLE 32)

TABLE 33: DISTRIBUTION OF CASES ACCORDING TO P53 GRADING IN TYPES OF RENAL CELL CARCINOMA

Types of RCC	P53 GRADE				
	0	**1+**	**2+**	**3+**	**TOTAL**
Clear cell RCC	15(55.6%)	8(29.6%)	4(14.8%)	0(0%)	27(100%)
Papillary RCC	2(86.6%)	2(86.6%)	3(42.9%)	0(0%)	7(100%)
Chromophobe RCC	0(0%)	0(0%)	2(100%)	0(0%)	2(100%)
Unclassified RCC	3(75%)	0(0%)	0(0%)	1(25%)	4(100%)
Total	20	10	9	1	40
Chi Square Test	P value -0.016				

In this study, 29.6 % of clear cell RCC showed p53 expression in the range of 1-10%. 55.6% of clear cell RCC were not immunoreactive for p53. 42.9% of papillary RCC showed p53 expression in the range of 11-90%. Only 2 chromophobe RCC studied which showed p53 expression in the range of 11-90%. 75% of unclassified tumors were not immunoreactive for p53. P value is significant between p53 expression and types of RCC. (TABLE 33)

TABLE 34: DISTRIBUTION OF CASES ACCORDING TO P53 GRADE IN CORRELATION WITH NUCLEAR GRADE

NUCLEAR GRADE	P53 GRADE				
	0	**1+**	**2+**	**3+**	**TOTAL**
Grade 1	6	1	0	0	7
Grade 2	5	3	3	0	11
Grade 3	4	5	3	0	12
Grade 4	5	1	3	1	10
Total	20	10	9	1	40
Chi-square test	P VALUE-0.347				

Among 7 grade 1 tumors, 6(85.7 %) were not immunoreactive for p53. In grade 2 tumors, 5(45.4 %) were not immunoreactive for p53expression. 6(54.5 %) of tumor showed p53 expression in the range of 1-90%. In grade 3 tumors 4(33.3 %) were not immunoreactive for p53expression. 5(41.7 %) of tumor showed p53 expression in the range of 1-10%. In grade 4 tumors, 5(50 %) were not immunoreactive for p53 expression. 4(30 %) of tumor showed p53 expression in the range of 11-90%. P value is found to be insignificant between p53 expression and nuclear grade. (TABLE 34)

TABLE 35 : DISTRIBUTION OF CASES ACCORDING TO P53 EXPRESSION IN RENAL CELL CARCINOMA

P53	TOTAL	Percentage
Positive	10	25%
Negative	30	75%
Total	40	100%

In this study, 30(75%) cases were negative for p53 expression. 10(25 %) cases were positive for p53 expression. (TABLE 35 & CHART 15).

TABLE 36 : DISTRIBUTION OF CASES ACCORDING TO P53 EXPRESSION IN TYPES OF RENAL CELL CARCINOMA

TYPES OF RCC	P53 POSITIVE	P53 NEGATIVE	TOTAL
Clear cell RCC	4(14.8%)	23(85.2%)	27(100%)
Papillary RCC	3(42.9%)	4(57.1%)	7(100%)
Chromophobe RCC	2(100%)	0(0%)	2(100%)
Unclassified RCC	1(25%)	3(75%)	4(100%)
Total	10	30	40(100%)
Chi Square Test	P VALUE-0.03		

In this study, out of 27 clear cell RCC cases, 23(85%) cases of clear cell RCC were negative for P53 expression. Of 7 papillary RCC studied, 4(57.1%) cases were negative for P53 and 42.9% were positive for P53. All 2 chromophobe RCC showed positivity for p53. 25% of unclassified type showed positivity for p53. (TABLE 36 & CHART 16)

TABLE 37: DISTRIBUTION OF CASES ACCORDING TO P53 EXPRESSION IN CORRELATION WITH NUCLEAR GRADE

NUCLEAR GRADE	P53 POSITIVE	P53 NEGATIVE	Total
Grade 1	0(0%)	7(100%)	7(100%)
Grade 2	3(27.3%)	8(72.7%)	11(100%)
Grade 3	3(25%)	9(75%)	12(100%)
Grade 4	4(40%)	6(60%)	10(100%)
Total	10	30	40
Chi-square test	P VALUE-0.313		

In this study, all RCC cases with nuclear grade1 were negative for p53 expression. In cases with nuclear grade 2 , 27.3% of cases were positive for p53. In cases with nuclear grade 3 tumors, 25% of cases were positive for p53. In cases with nuclear grade 4 tumors, 40% of them were positive for p53. Expression of p53 did not correlate with nuclear grade. (TABLE 37 & CHART 16)

CORRELATION OF P53 EXPRESSION WITH VARIOUS CLINICO PATHOLOGICAL FACTORS

TABLE 38 :CORRELATION OF AGE WITH P53 EXPRESSION IN RCC

AGE	P53 POSITIVE	P 53 NEGATIVE	TOTAL
≤50	7	15	22
>50	3	15	18
Chi-Square Tests	P Value-0.271		

In this study, 7cases were positive for P53 in the age of less than or equal to 50 years. 3 cases were positive for P53 in the age group more than 50 years. There is no correlation between age and P53expression. (TABLE 38)

TABLE 39 :CORRELATION OF SEX WITH P53 EXPRESSION IN RCC

SEX	P53 POSITIVE	P 53 NEGATIVE	TOTAL
Male	6	22	28
Female	4	8	12
Chi-Square Tests	P value-0.426		

In this study, 6 males were positive for P53 expression and 4 females were positive for P53. There is no correlation between sex and P53 expression. (TABLE 39)

TABLE 40 :CORRELATION OF SIZE WITH P53 EXPRESSION IN RCC

SIZE	P53 POSITIVE	P53 NEGATIVE	TOTAL
≤5cm	1	8	9
>5 cm	9	22	31
Chi-Square Tests	P VALUE-0.274		40

In this study, 22 tumors were of size more than 5 cm; among this, 9 were positive for P53 and one tumor with size less than or equal to 5cm positive for P53. There is no correlation between size of the tumor and P53 expression. (TABLE 40)

TABLE 41 :CORRELATION OF STAGE WITH P53 EXPRESSION IN RCC

STAGE	P53 POSITIVE	P53 NEGATIVE	TOTAL
1	2	12	14
2	2	7	9
3	4	8	12
4	2	3	5
Chi-Square Tests	P value-0.585		40

In this study, stage 3 cases had maximum P53 expression. There is no significant correlation between stage and P53 expression. (TABLE 41)

TABLE 42 : DISTRIBUTION OF CASES ACCORDING TO MUC1 GRADING IN RENAL CELL CARCINOMA

MUC1 GRADE	TOTAL
0	1(2.5%)
1(<10%)	4(10 %)
2(10-25%)	3(7.5 %)
3(25-50%)	5(12.5 %)
4(50-75%)	7(17.5 %)
5(75-90%)	13(32.5 %)
6>90%	7(17.5 %)
TOTAL	40(100 %)

In this study, 32.5% of RCC expressed MUC1 in the range of 75-90%. Maximum expression of MUC1 was seen in 17.5% of RCC. Grade 2 expression was seen in 7.5% of cases. 2.5% of cases did not take up the stain. (TABLE 42)

TABLE 43 : DISTRIBUTION OF CASES ACCORDING TO MUC1 GRADING IN TYPES OF RENAL CELL CARCINOMA

Types of RCC	MUC1 GRADE							
	0	**1 <10%**	**2 (10-25%)**	**3 (25-50%)**	**4 (50-75%)**	**5 (75-90%)**	**6 >90%**	**Total**
Clear cell RCC	1 3.7%	3 11.1%	2 7.4%	4 14.8%	3 11.1%	9 22.5 %	5 18.6%	27 100%
Papillary RCC	0 0%	1 14.3%	0 0%	1 14.3%	2 28.5%	2 28.6%	1 14.3%	7 100%
Chromo phobe RCC	0 0%	0 0%	1 50%	0 0%	1 50%	0 0%	0 0%	2 100%
Unclassified	0 0%	0 0%	0 0%	0 0%	1 25%	2 50%	1 25%	4 100%
Total	1	4	3	5	7	13	7	40
Chi Square Test	**P value-0.856**							

In clear cell RCC, maximum cases(22.5%) expressed MUC1 in the range of 75-90%. Maximum expression (>90%) is seen in 18.5% cases of clear cell RCC. Lowest expression (<10%) is seen in 11.1% of clear cell RCC.

In papillary RCC, 57.2% of cases expressed MUC1 in the range of 50-90%. In chromophobe RCC, expression of MUC1 ranged from 10-75 %. In unclassified type, the expression of MUC1 is in the range of 25-100 %. (TABLE 43)

TABLE 44 : DISTRIBUTION OF CASES ACCORDING TO MUC1 GRADING IN RENAL CELL CARCINOMA IN CORRELATION WITH NUCLEAR GRADE

NUC LEAR GRADE	MUC1 GRADE							
	0	**1 <10%**	**2 (10-25%)**	**3 (25-50%)**	**4 (50-75%)**	**5 (75-90%)**	**6 >90%**	**Total**
1	1 14.3%	2 28.6%	1 14.3%	0 0%	1 14.3%	1 14.3%	1 14.3%	7 100%
2	0 0%	2 28.6%	1 9.0%	4 36.4%	2 18.2%	2 18.2%	0 0%	11 100%
3	0 0%	0 0%	1 8.3%	1 8.3%	4 33.3%	5 41.7%	1 8.3%	12 100%
4	0 0%	0 0%	0 0%	0 0%	0 0%	5 50%	5 50%	10 100%
TOTAL	0	4	3	5	7	13	7	40
Chi Square Test	P VALUE-0.015							

- In this study, 28.6% of grade 1 RCC cases showed <10% MUC1 expression.
- 36.4% of grade 2 RCC cases showed MUC1 expression in the range of 25-50%.
- In grade 3 RCC, 41.7% cases showed MUC1 expression in the range of 75-90%
- In grade 4 RCC, 100% cases showed MUC1 expression in the range of 75-100%.

- In this study, when nuclear grade is increased, expression of MUC1 is also increased. (TABLE 44)

TABLE 45 : DISTRIBUTION OF CASES ACCORDING TO MUC1 EXPRESSION IN RENAL CELL CARCINOMA

MUC1	TOTAL	PERCENTAGE
POSITIVE	35	87.5%
NEGATIVE	5	12.5%
TOTAL	40	100%

In this study, 87.5% cases of RCC cases were positive for MUC1. 12.5% cases were negative for MUC1 expression. (TABLE 45 & CHART 18)

TABLE 46 : DISTRIBUTION OF CASES ACCORDING TO MUC1 EXPRESSION IN TYPES OF RENAL CELL CARCINOMA

TYPES OF RCC	MUC1 POSITIVE	MUC1 NEGATIVE	TOTAL
Clear cell RCC	23	4	27
Papillary RCC	6	1	7
Chromophobe RCC	2	0	2
Unclassified RCC	4	0	4
Total	35	5	40
Chi Square Test	P VALUE-0.799		

In this study, 23(85%) cases of clear cell RCC were positive for MUC1 expression. In papillary RCC, out of 7 cases, 6 were positive for MUC1 expression.

All 2 chromophobe cases showed positive MUC1 expression. All 4 unclassified cases showed positive MUC1 expression. (TABLE 46 & CHART 19)

TABLE 47 : DISTRIBUTION OF CASES ACCORDING TO MUC1 EXPRESSION IN RENAL CELL CARCINOMAIN CORRRELATION WITH NUCLEAR GRADE

NUCLEAR GRADE	MUC1 POSITIVE	MUC1 NEGATIVE	TOTAL
Grade 1	4(57.2%)	3(42.8%)	7(100%)
Grade 2	9(81.8%)	2(18.1%)	11(100%)
Grade 3	12(100%)	0(0%)	12(100%)
Grade 4	10(100%)	0(0%)	10(100%)
Total	35	5	40
Chi-square test	P value-0.025		

- In this study in RCC with nuclear grade 1, 57.2% cases were positive for MUC1 expression.
- In RCC with nuclear grade 2, 81.8 % cases were positive for MUC1 expression.

- In RCC with nuclear grade 3, 100% cases were positive for MUC1 expression.
- In RCC with nuclear grade 4, 100% cases were positive for MUC1 expression.
- There is a positive correlation between expression of MUC1 and nuclear grade. (TABLE 47 & CHART 20)

TABLE 48 :CORRELATION OF AGE WITH MUC1 EXPRESSION IN RCC

AGE	MUC1 POSITIVE	MUC1 NEGATIVE	TOTAL
<=50	19	3	22
>50	16	2	18
CHI-SQUARE TEST	P value-0.810		40

In this study, 19 cases were positive for MUC1 in the age less than 50 years. 16 cases were positive for MUC1 in the age more than 50 years. There is no correlation between age of presentation and MUC1 expression. (TABLE 48)

TABLE 49 :CORRELATION OF SEX WITH MUC1 EXPRESSION IN RCC

SEX	MUC1 POSITIVE	MUC1 NEGATIVE	TOTAL
Male	25	3	28
Female	10	2	12
CHI-SQUARE TEST	P VALUE-0.602		40

In this study, 25 males were positive for MUC1. 10 females were positive for MUC1. There is no correlation between sex and MUC1 expression. (TABLE 49)

TABLE 50 :CORRELATION OF SIZE WITH MUC1 EXPRESSION IN RCC

SIZE	MUC1 POSITIVE	MUC1 NEGATIVE	TOTAL
<=5	8	2	10
>5	27	3	30
CHI-SQUARE TEST	P VALUE-0.408		40

In this study, 27 tumors positive for MUC1were more than 5 cm. 8 tumors positive for MUC1were less than or equal to 5 cm. There is no correlation between size of tumor and MUC1 expression. (TABLE 50)

TABLE 51 :CORRELATION OF STAGE WITH MUC1 EXPRESSION IN RCC

STAGE	MUC1 POSITIVE	MUC1 NEGATIVE	TOTAL
1	9	5	14
2	9	0	9
3	12	0	12
4	5	0	5
CHI-SQUARE TEST	P VALUE -0.014		40

In this study, 9 stage 1 cases were positive for MUC1 and 5 cases were negative. In stage 2, 3 and 4, all cases were positive for MUC1. There is statistically significant correlation between stage at presentation and MUC1 expression. (TABLE 51)

CHARTS AND PICTURES

CHART 1: DISTRIBUTION OF TYPE OF RENAL CELL CARCINOMA

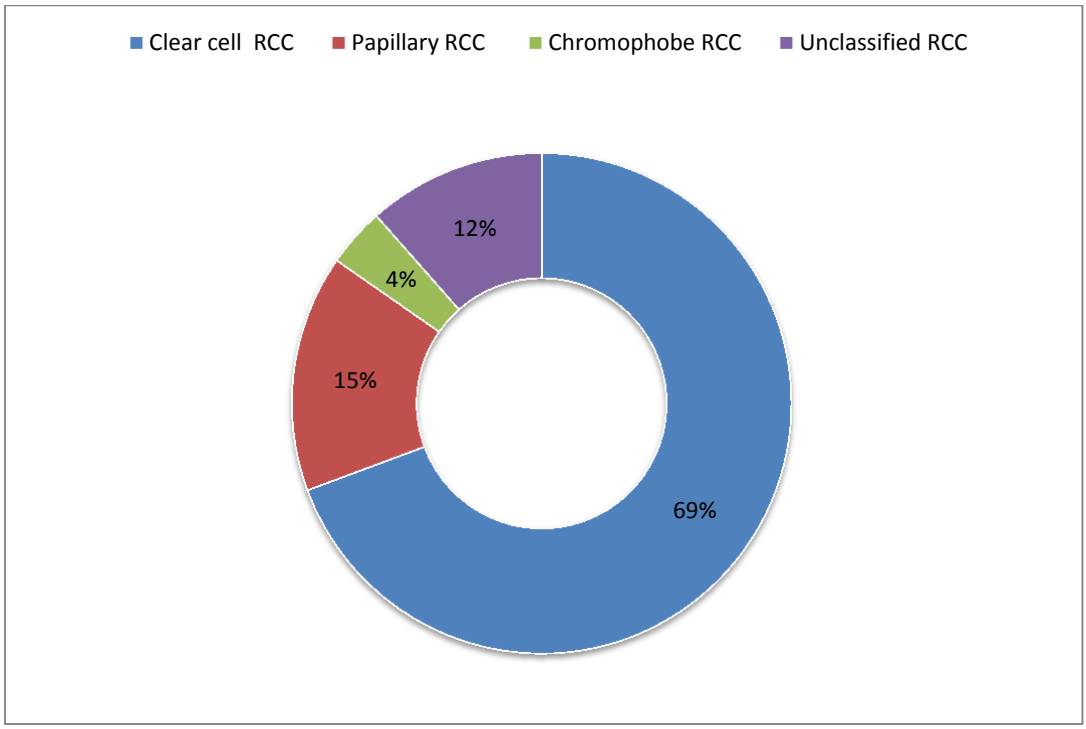

CHART 2 : AGE WISE DISTRIBUTION OF RENAL CELL CARCINOMA

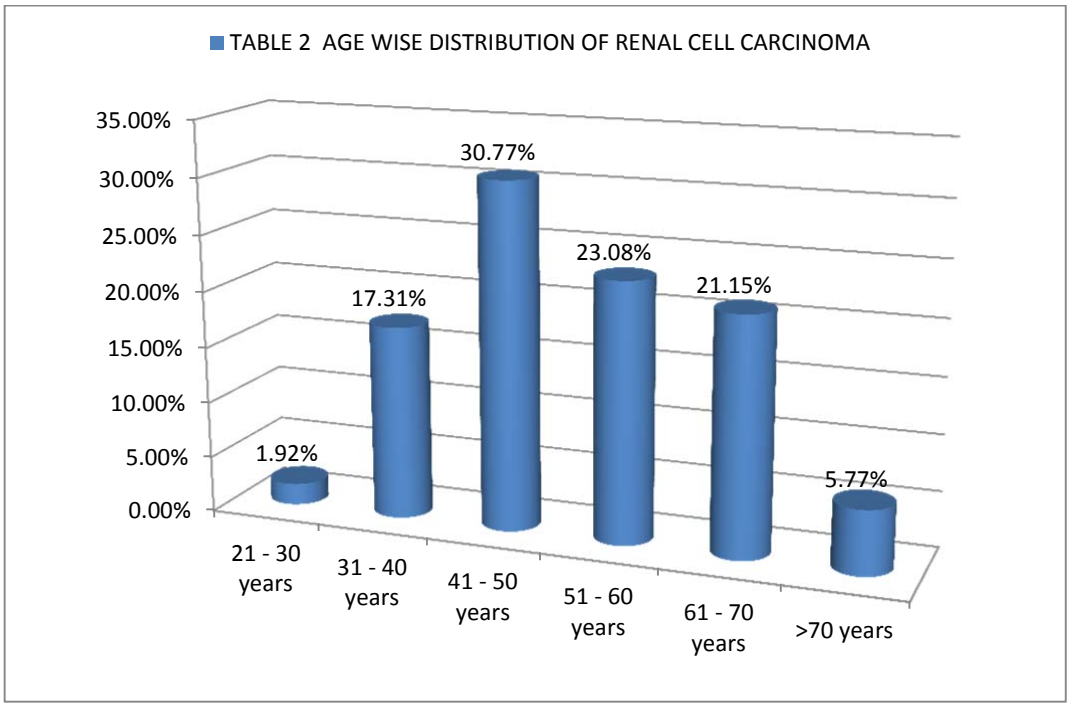

CHART 3: SEX WISE DISTRIBUTION OF RENAL CELL CARCINOMA

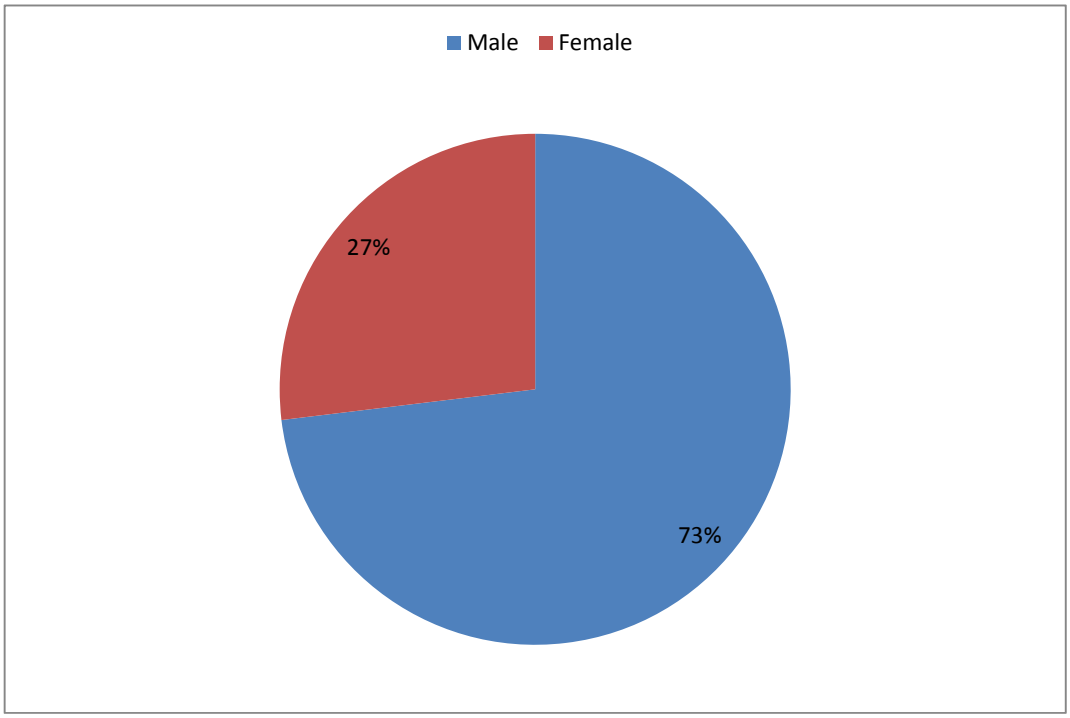

CHART 4: SIDE WISE DISTRIBUTION OF RENAL CELL CARCINOMA

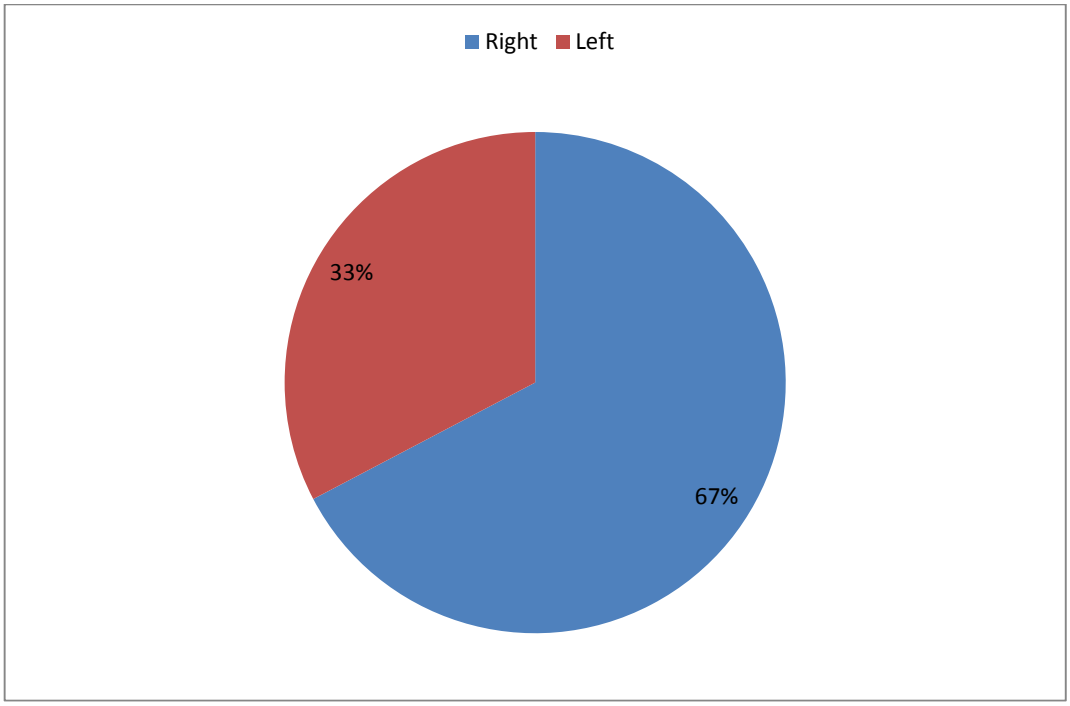

CHART 5: DISTRIBUTION OF RENAL CELL CARCINOMA BASED ON THE SIZE

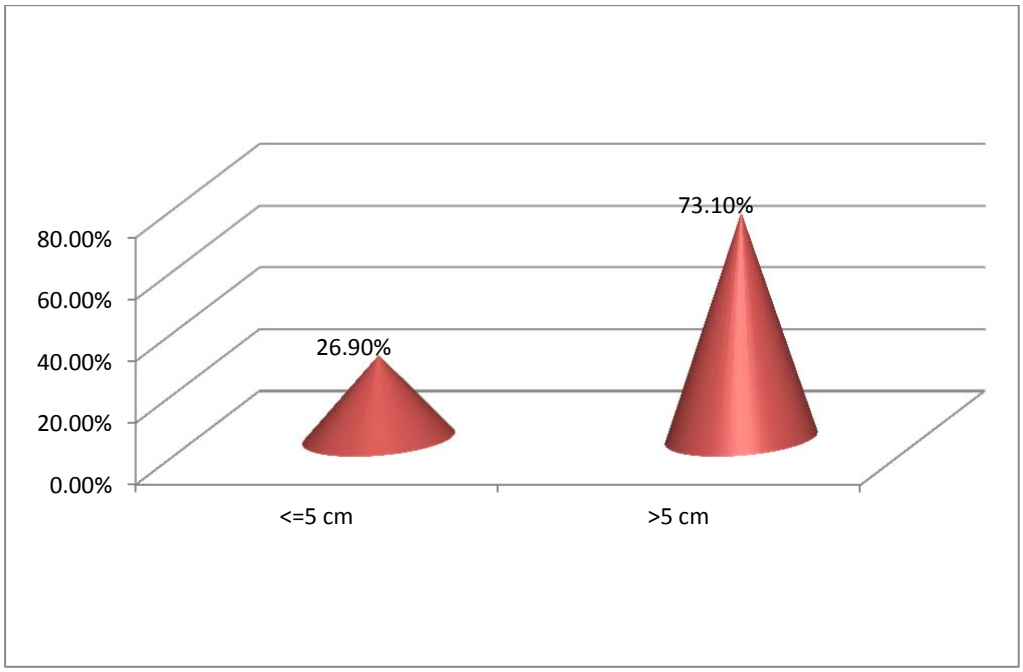

CHART 6: DISTRIBUTION OF TYPES OF RENAL CELL CARCINOMA BASED ON THE SIZE

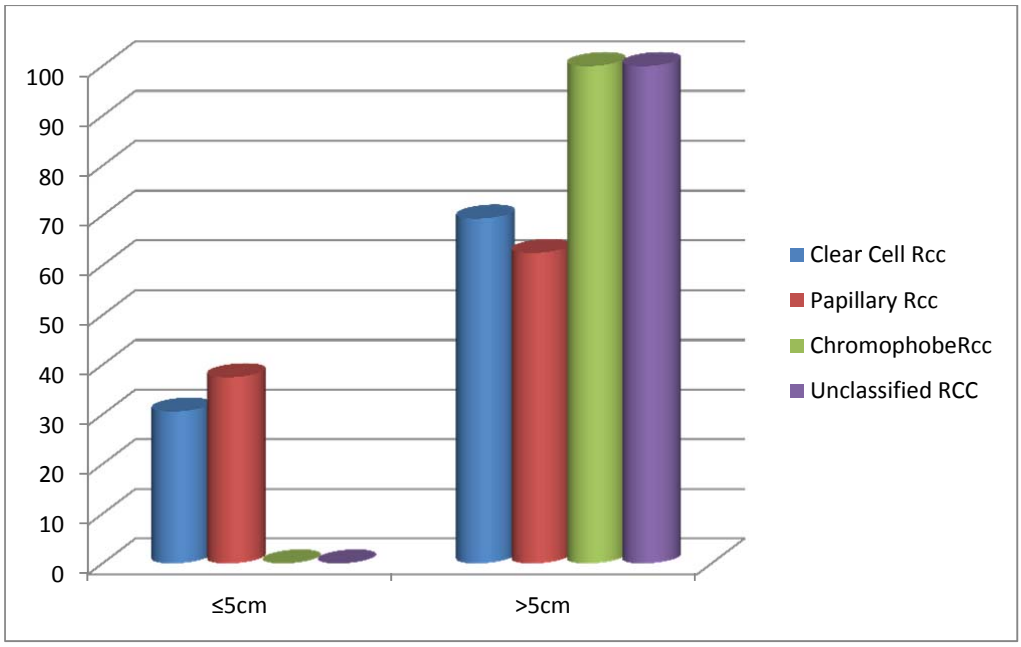

CHART 7: GRADE AT PRESENTATIONAMONG RENAL CELL CARCINOMA

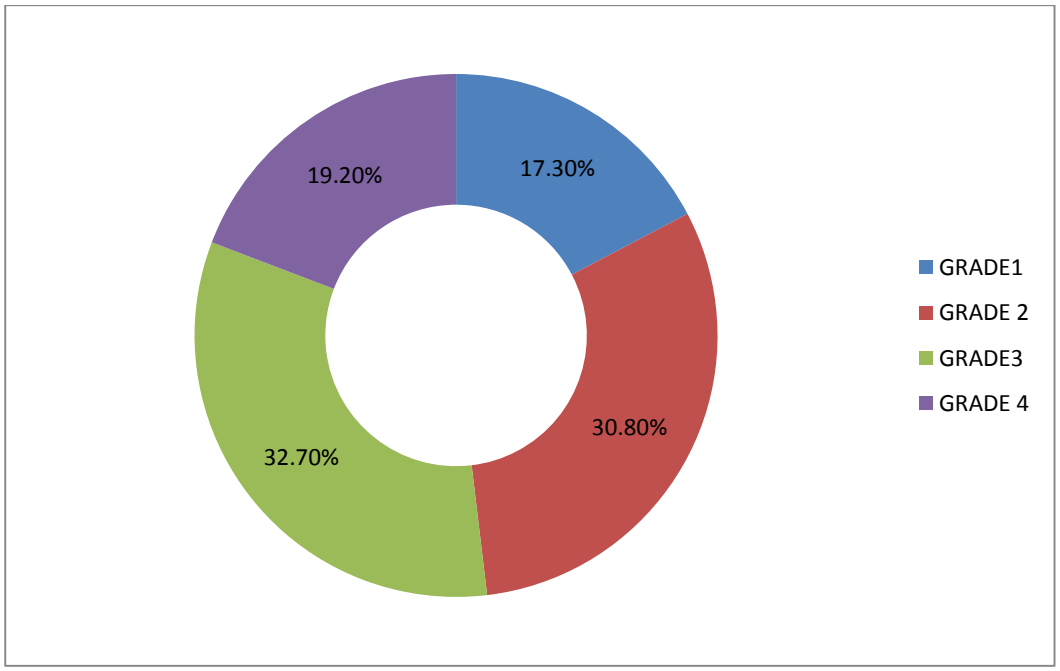

CHART 8: GRADE AT PRESENTATION AMONG TYPES OF RENAL CELL CARCINOMA

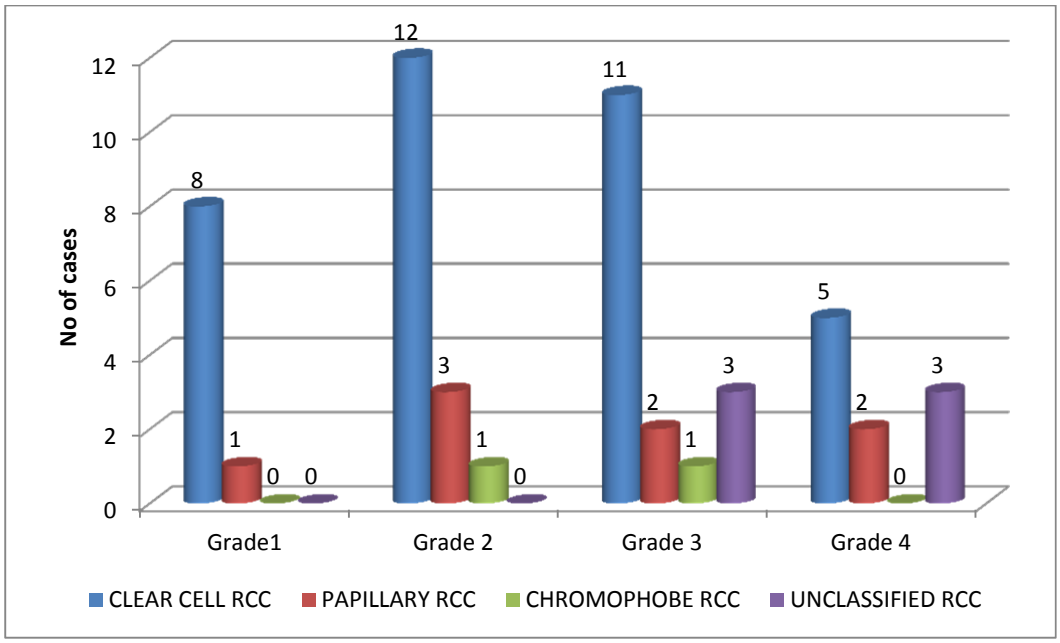

CHART 9: Tstage AT PRESENTATIONAMONG RENAL CELL CARCINOMA

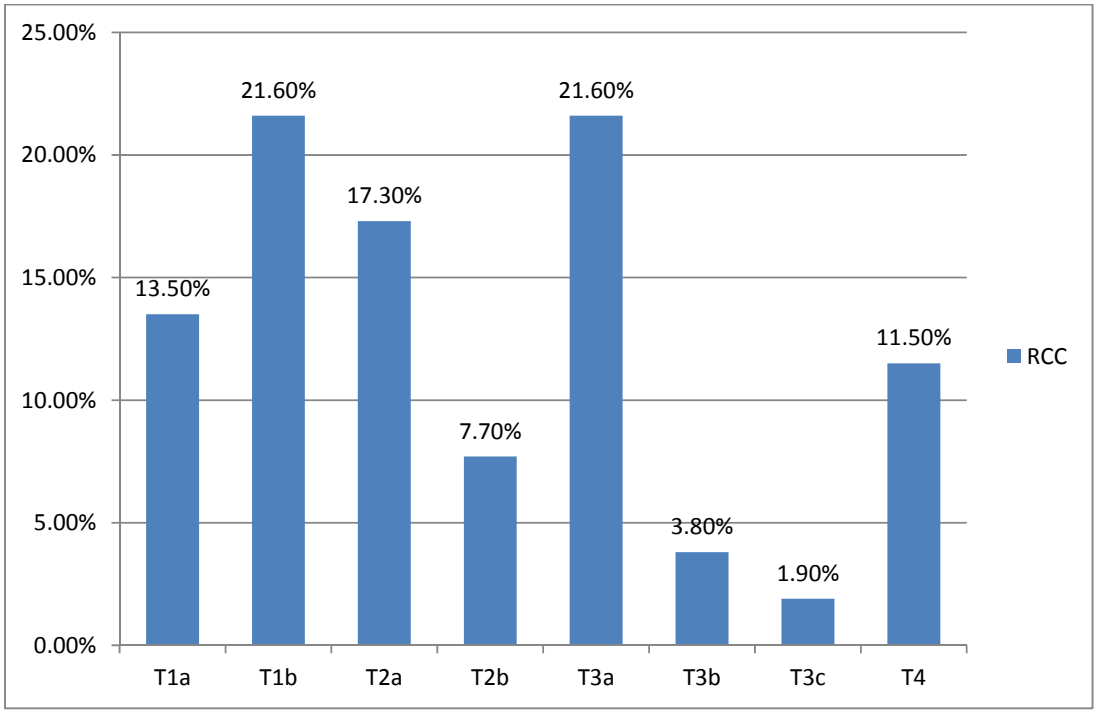

CHART 10: STAGE AT PRESENTATION AMONG TYPES OF RENAL CELL CARCINOMA

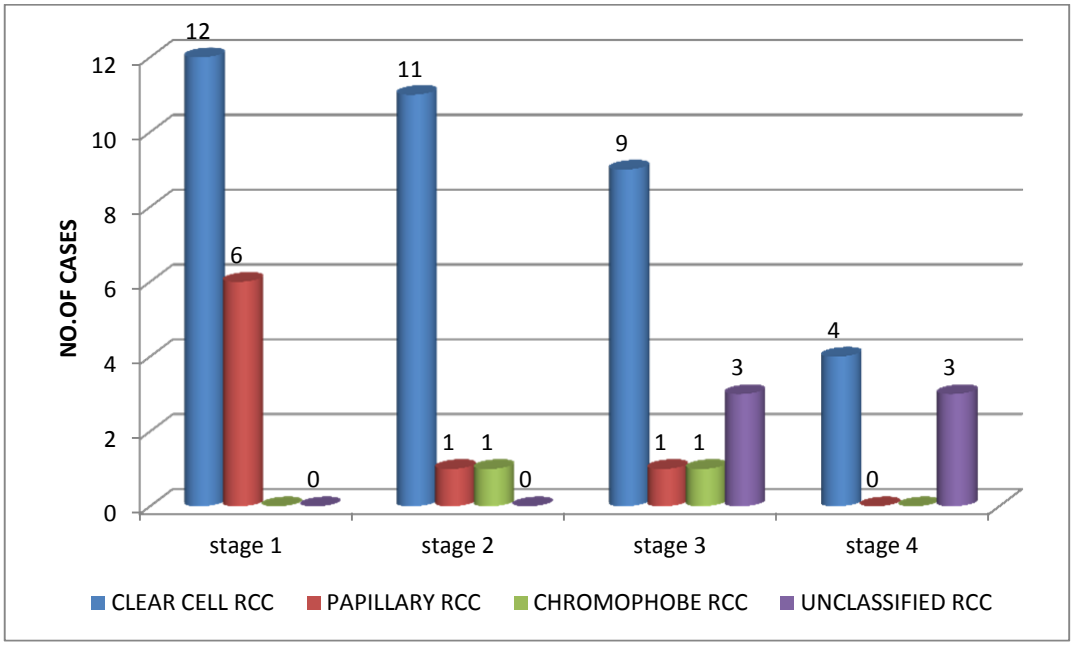

CHART 11:DISTRIBUTION OF CASES ACCORDING TO KI 67 EXPRESSION

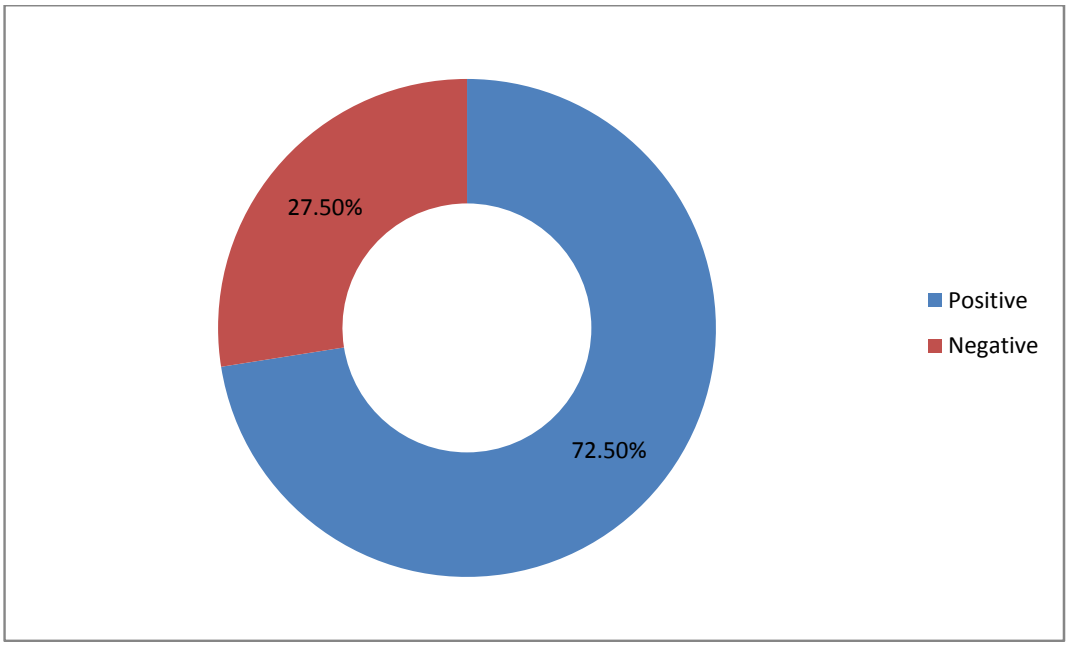

CHART 12 : DISTRIBUTION OF CASES ACCORDING TO KI67 EXPRESSION IN TYPES OF RENAL CELL CARCINOMA

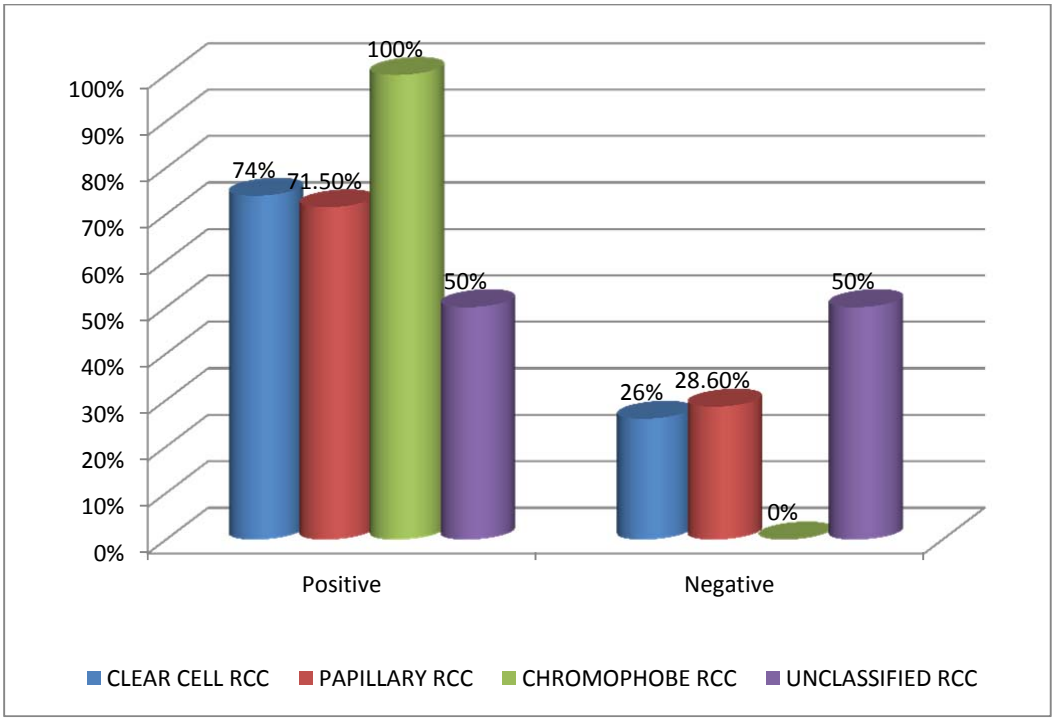

CHART 13: DISTRIBUTION OF CASES ACCORDING TO KI67 EXPRESSION IN CORRELATION WITH NUCLEAR GRADE

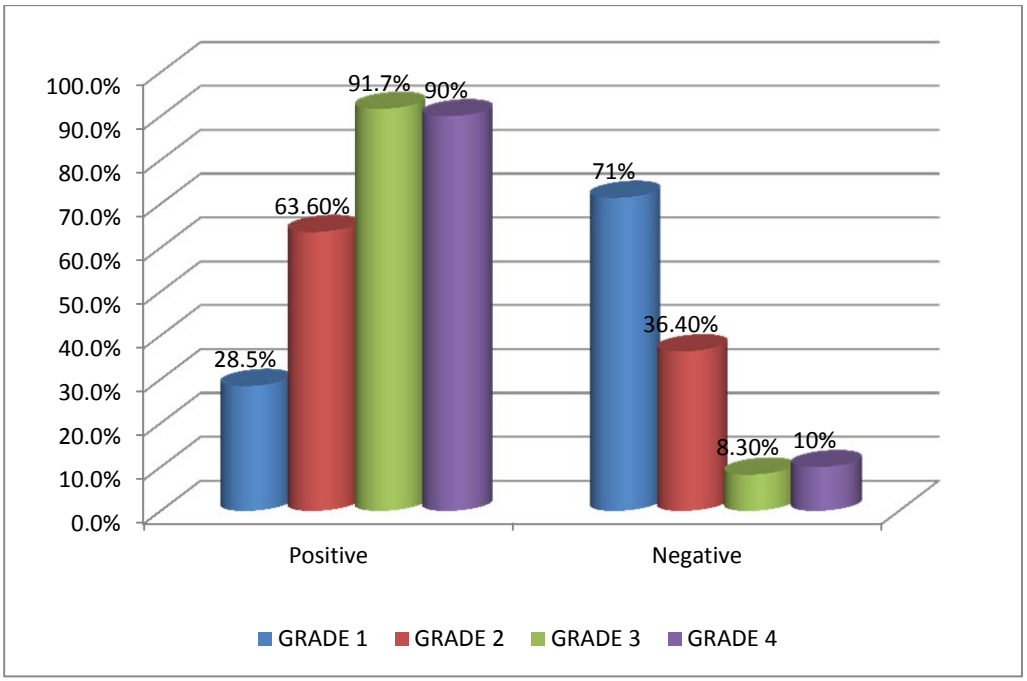

CHART 14: DISTRIBUTION OF CASES ACCORDING TO MEAN KI67 LABELLING INDEX IN CORRELATION WITH NUCLEAR GRADE

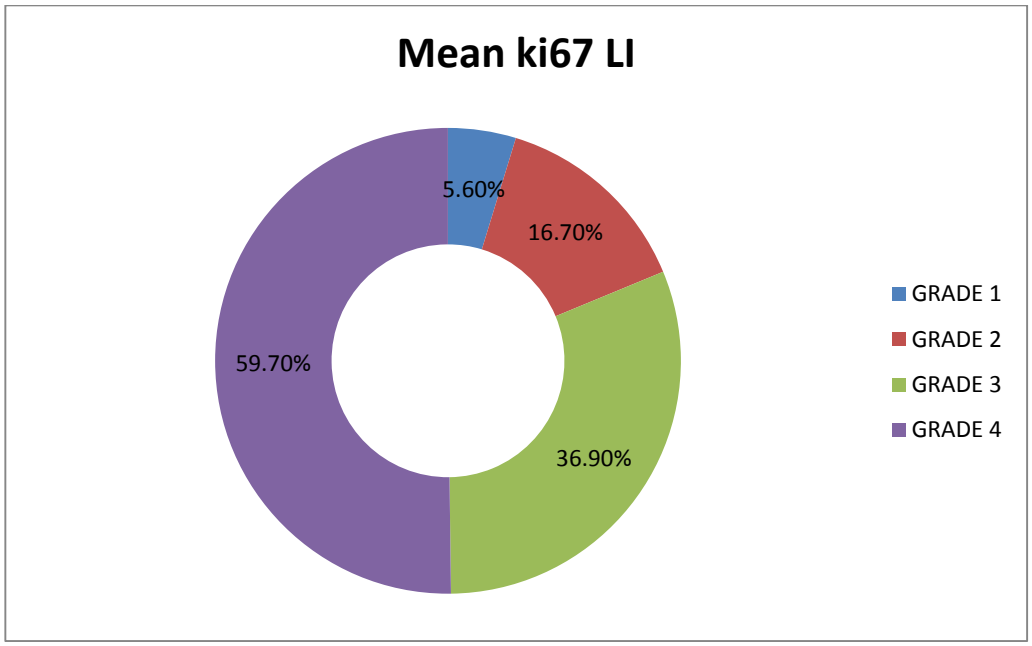

CHART 15 : DISTRIBUTION OF CASES ACCORDING TO P53 EXPRESSION IN RENAL CELL CARCINOMA

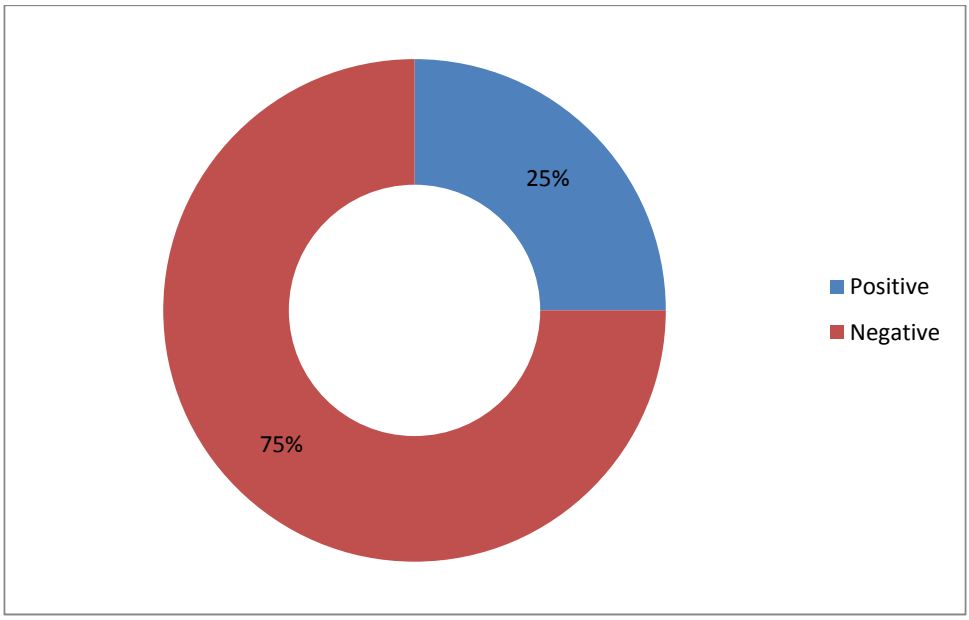

CHART 16: DISTRIBUTION OF CASES ACCORDING TO P53 EXPRESSION IN TYPES OF RENAL CELL CARCINOMA

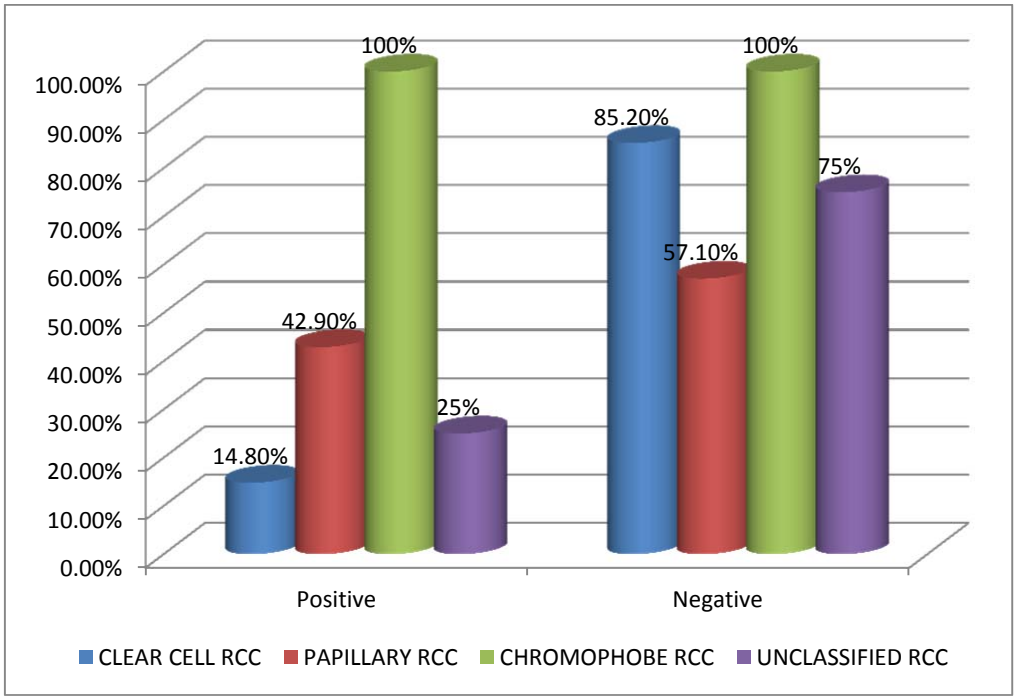

CHART 17 : DISTRIBUTION OF CASES ACCORDING TO P53 EXPRESSION IN CORRELATION WITH NUCLEAR GRADE

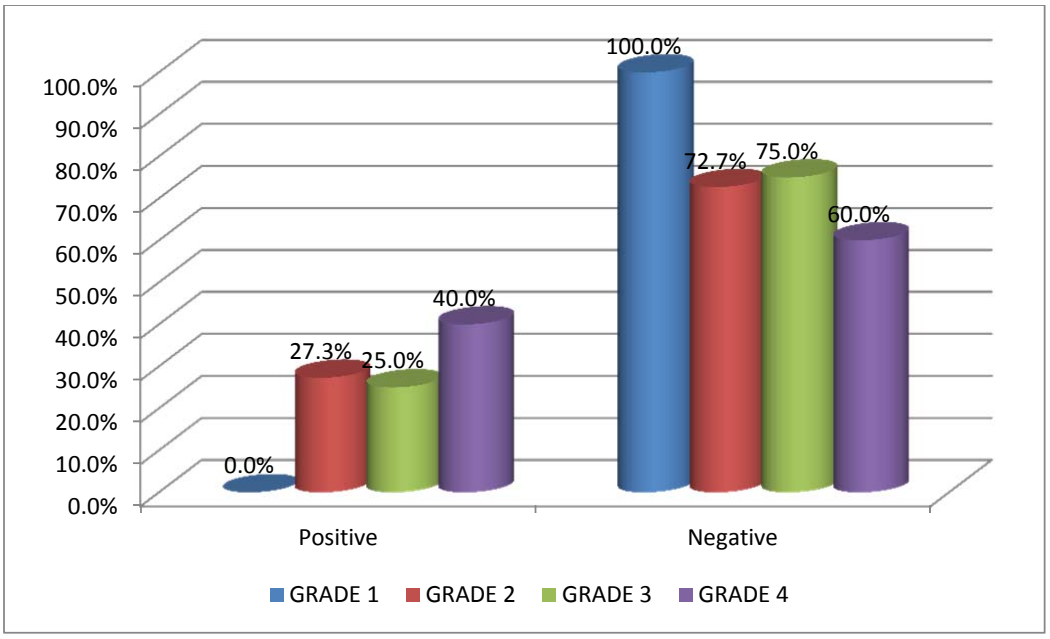

CHART 18 : DISTRIBUTION OF CASES ACCORDING TO MUC1 EXPRESSION IN RENAL CELL CARCINOMA

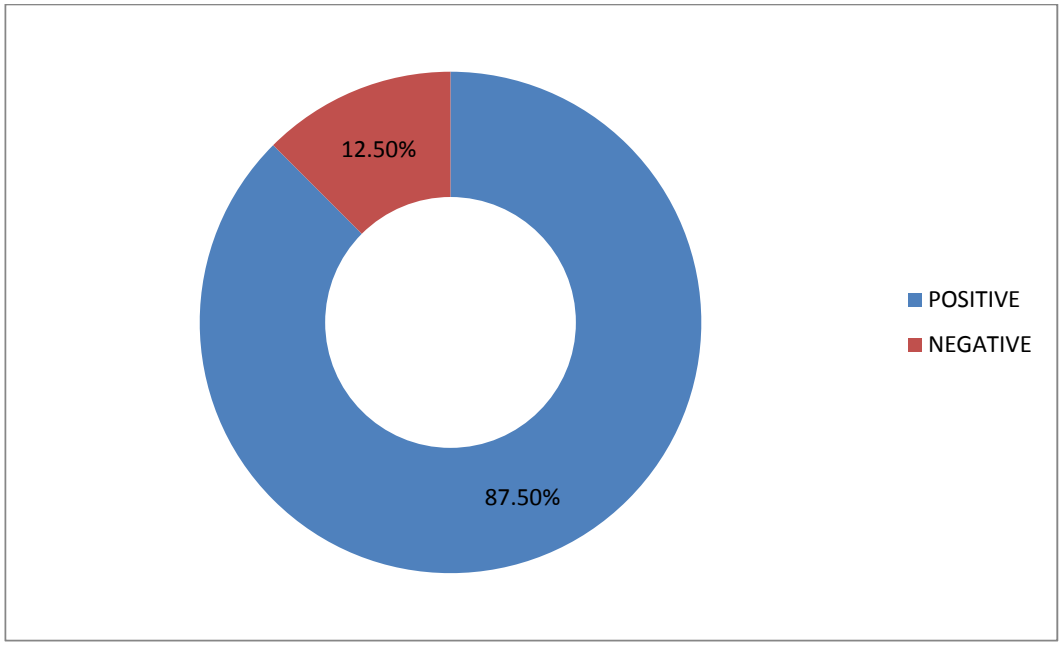

CHART 19 : DISTRIBUTION OF CASES ACCORDING TO MUC1 EXPRESSION IN TYPES OF RENAL CELL CARCINOMA

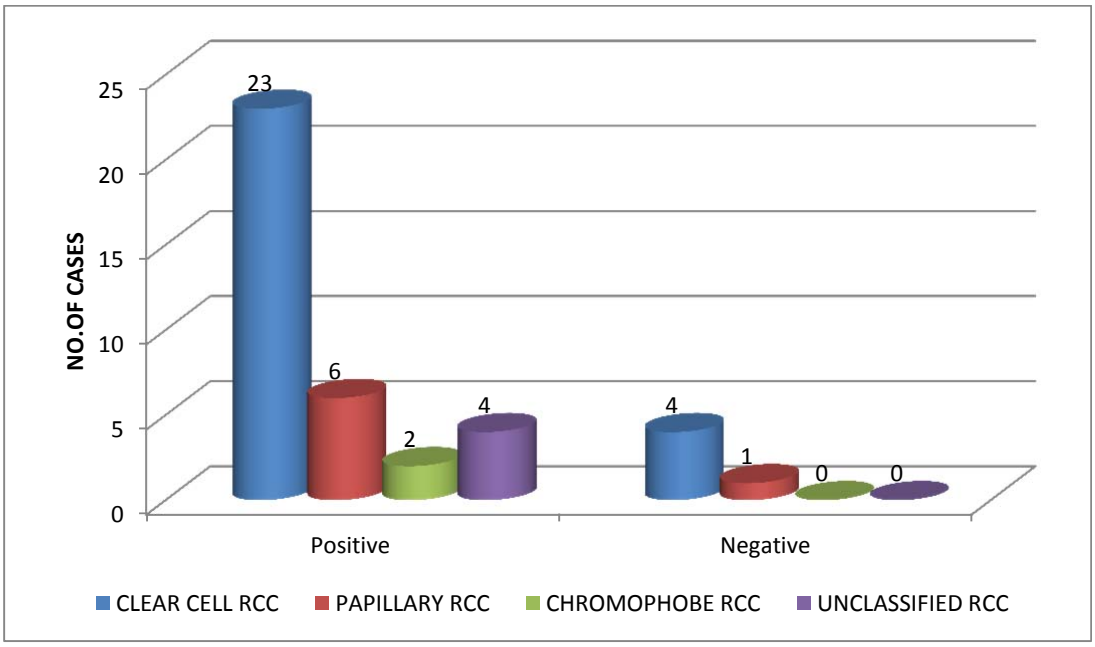

CHART 20 : DISTRIBUTION OF CASES ACCORDING TO MUC1 EXPRESSION IN RENAL CELL CARCINOMAIN CORRRELATION WITH NUCLEAR GRADE

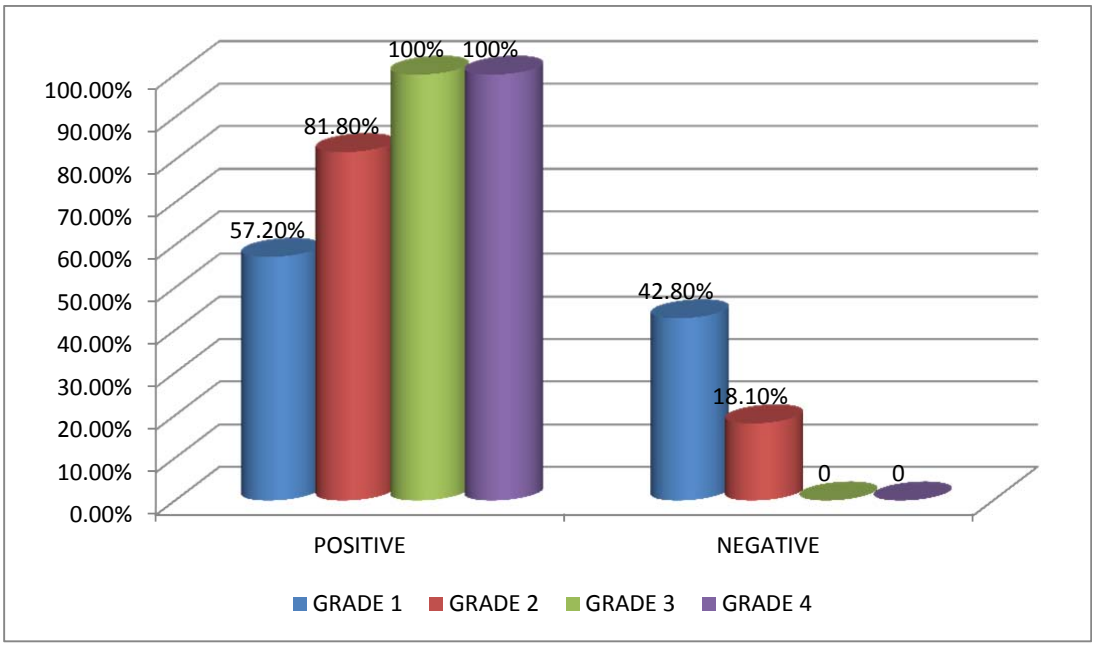

Figure 1:Clear cell renal cell carcinoma

Figure 2: papillary renal cell carcinoma

Figure 3:chromophobe renal cell carcinoma

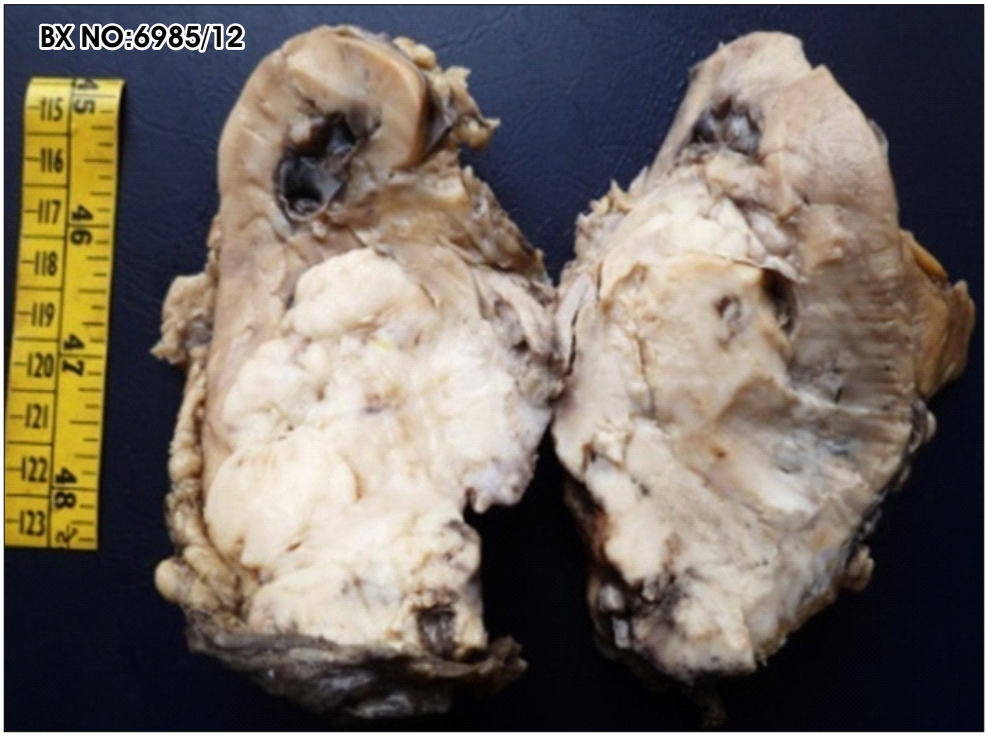

Figure 4: Unclassified RCC

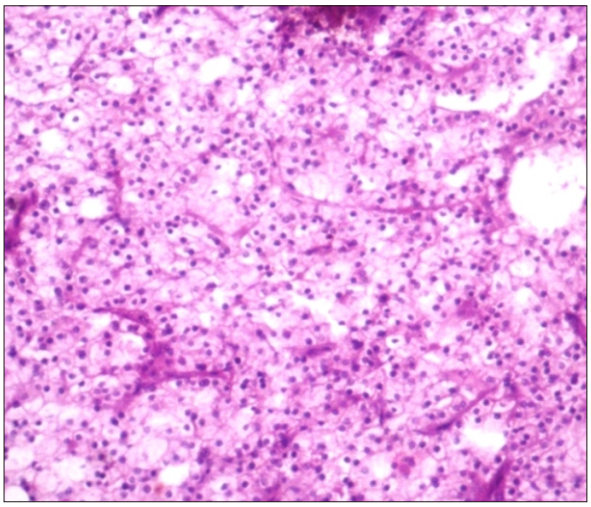

Figure 5: Clear cell RCC-Nuclear grade 1 (100x)

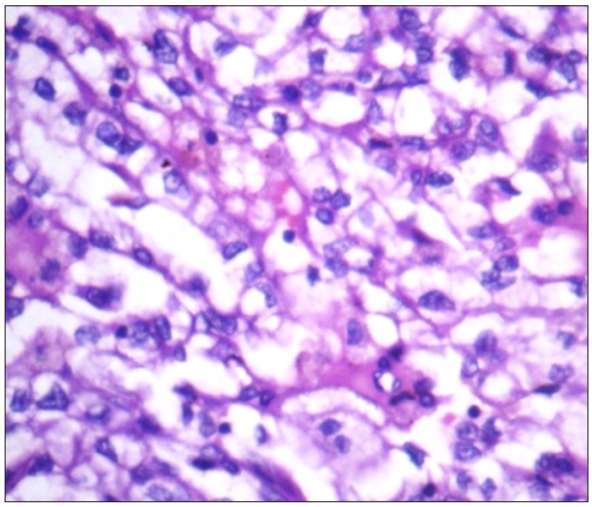

Figure 6:Clear cell RCC-Nuclear grade 2 (400x)

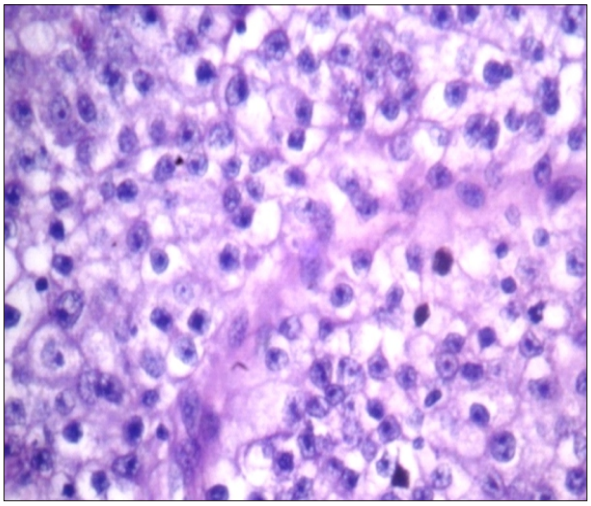

Figure 7: Clear cell RCC-Nuclear grade 3 (400x)

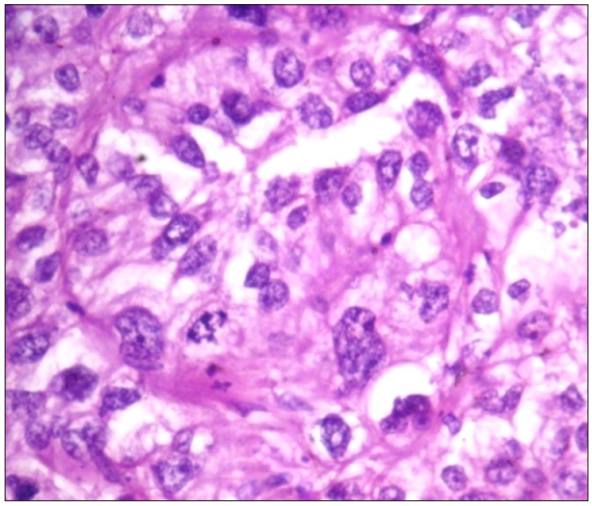

Figure 8: Clear cell RCC-Nuclear grade 4 (400x)

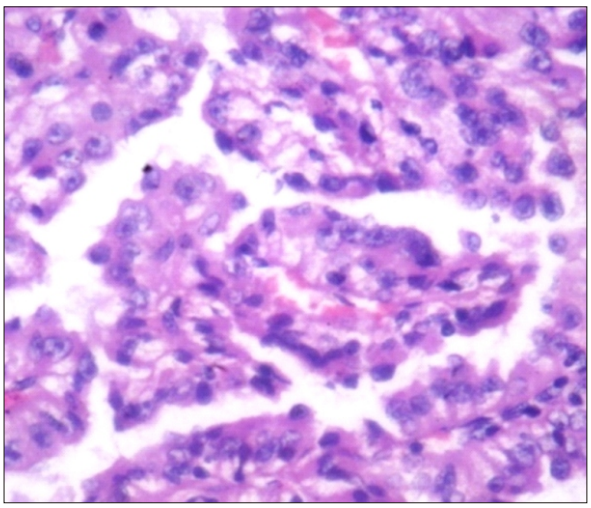

Figure 9:papillary RCC- type 1 nuclear grade 3 (400x)

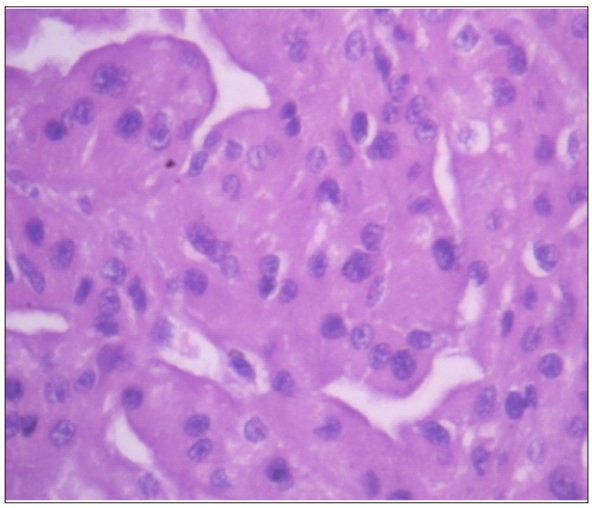

Figure 10: Chromophobe RCC-nuclear grade -3(400x)

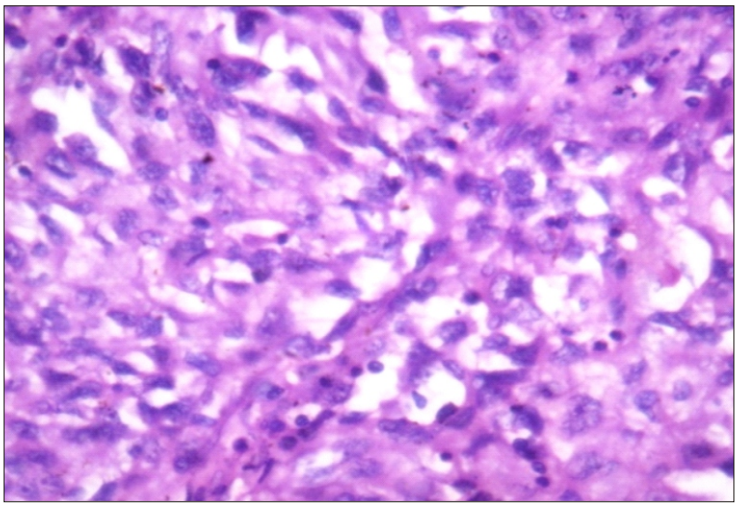

Figure 11:Unclassified RCC-Nuclear grade-4 (400x)

Ki67

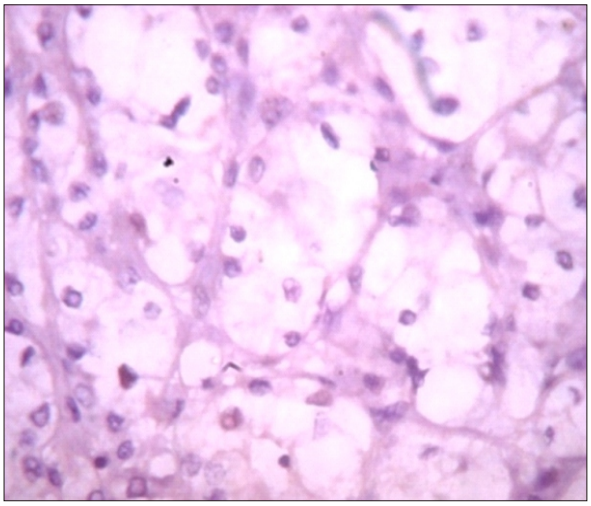

Figure 12: Clear cell RCC Nuclear grade 1 - negative for KI67(400x)

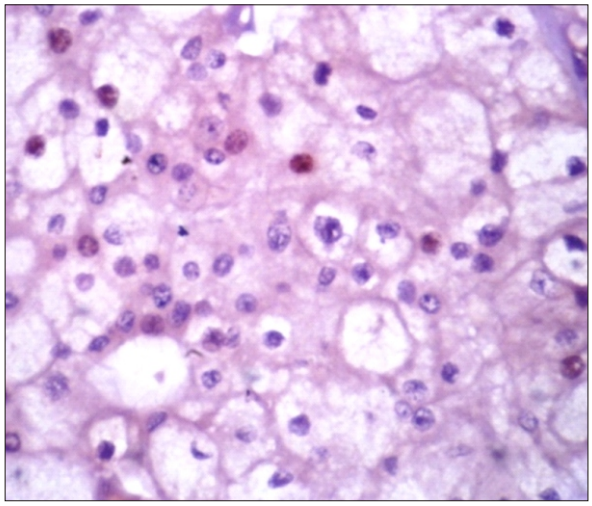

Figure 13: Clear cell RCCNuclear grade 2-KI67 LI-16%(400x)

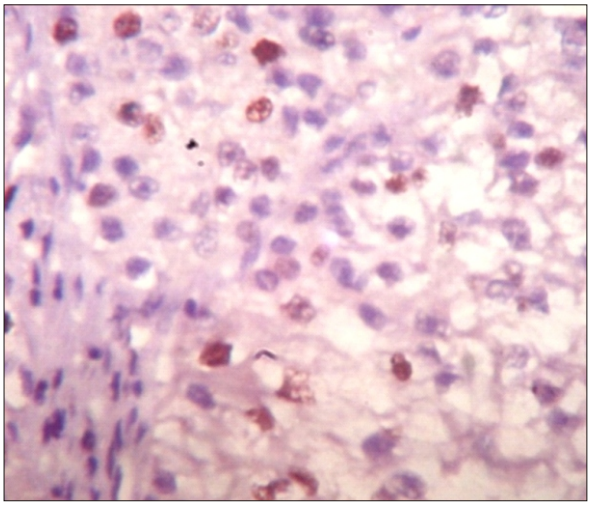

Figure 14: Clear cell RCC- Nuclear grade 3 -KI67 LI-30%(400x)

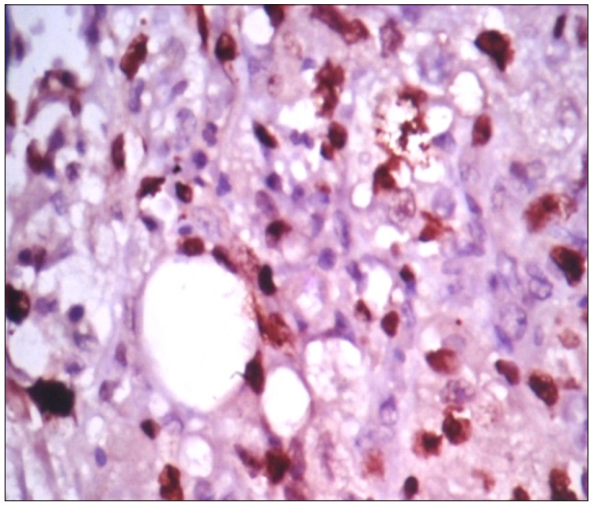

Figure 15: 8386/13 Clear cell RCC- Nuclear grade 4 -KI67 LI-70%(400x)

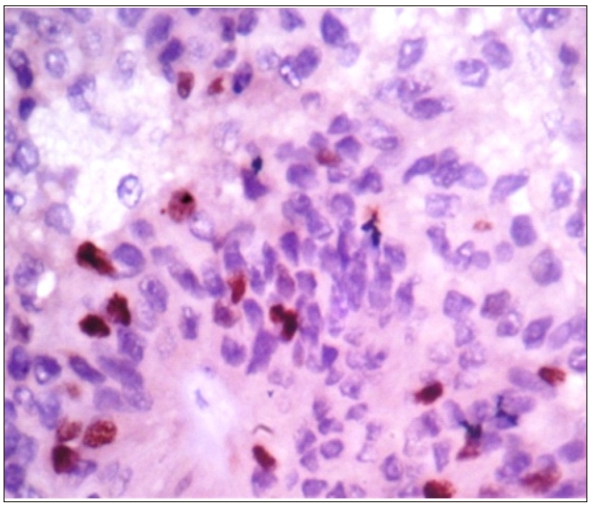

Figure 16: Papillary RCC- Nuclear grade 2 KI67 LI-16%(400x)

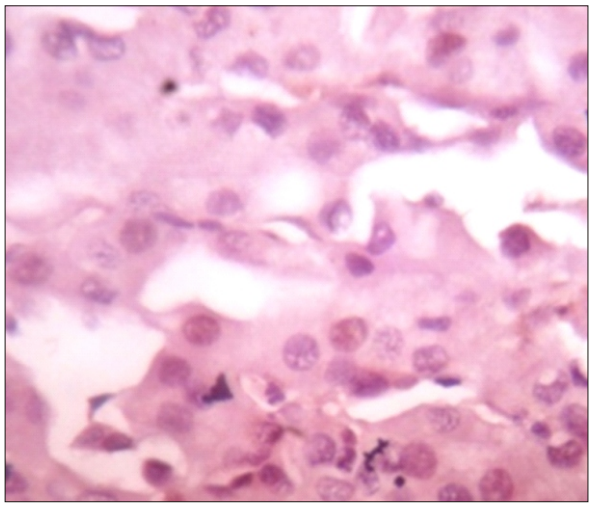

Figure 17: ChromophobeRCC- Nuclear grade 2- KI67 LI-15%(400x)

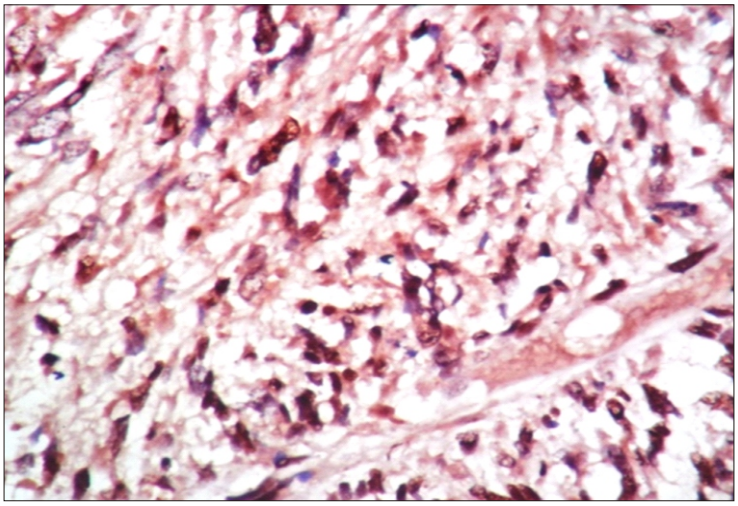

Figure 18: Unclassified RCC- Nuclear grade 4-KI67 LI-70%(400x)

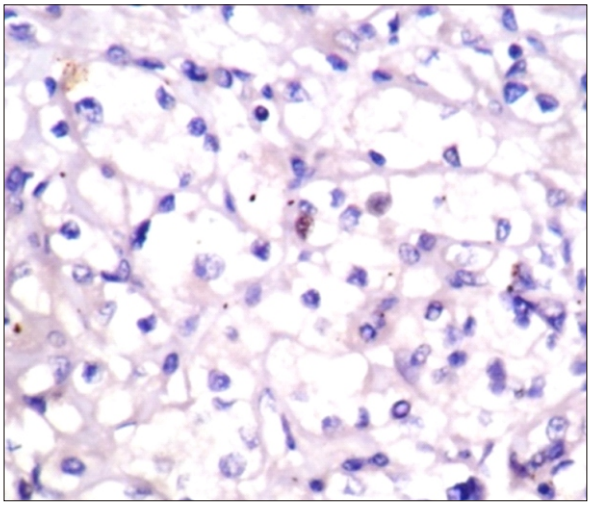

Figure 19: Clear cell RCC-Nuclear grade 1 -Negative for p53(400x)

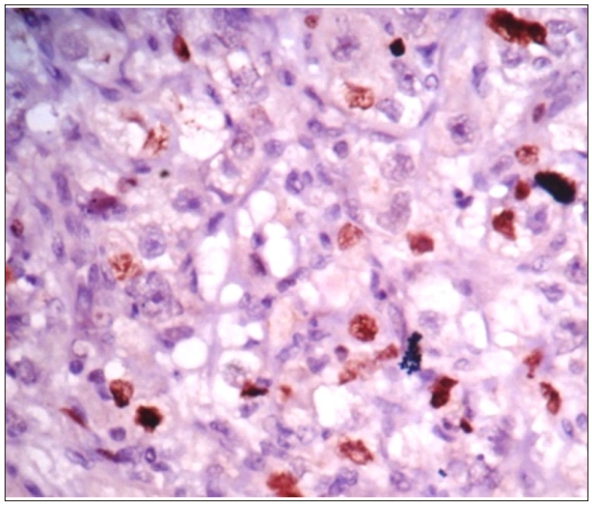

Figure 20: Medium level expression of p53 in Clear cell RCC-Nuclear grade 4 (400x)

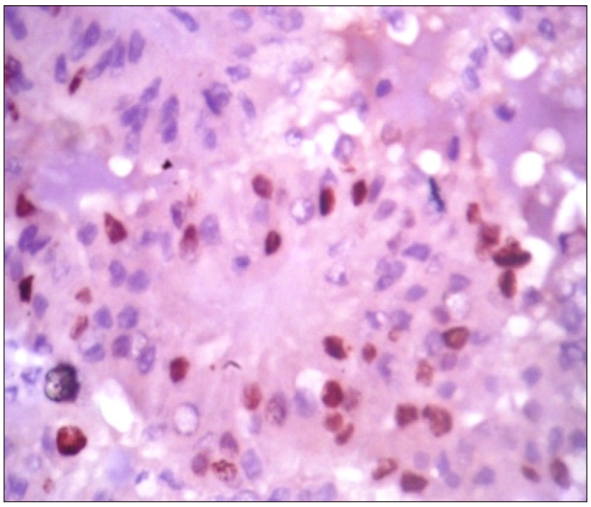

Figure 21: Medium level expression of p53 in papillary RCC-grade 2(400x)

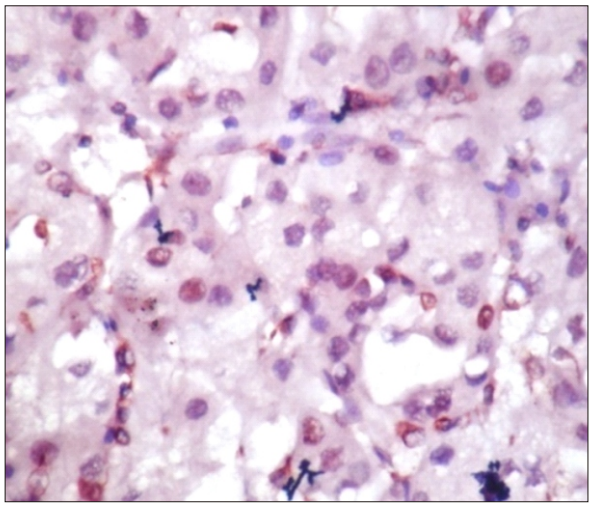

Figure 22: Medium level expression of p53 in Chromophobe RCC-Nuclear grade 2 (400x)

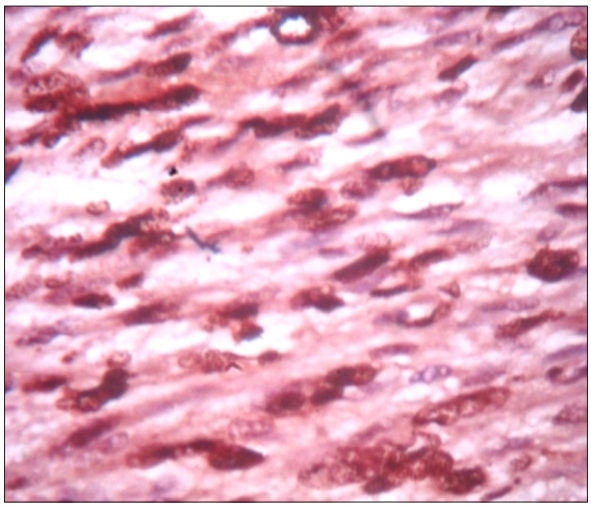

Figure 23: High level expression of p53 in Unclassified RCC-grade 4(400x)

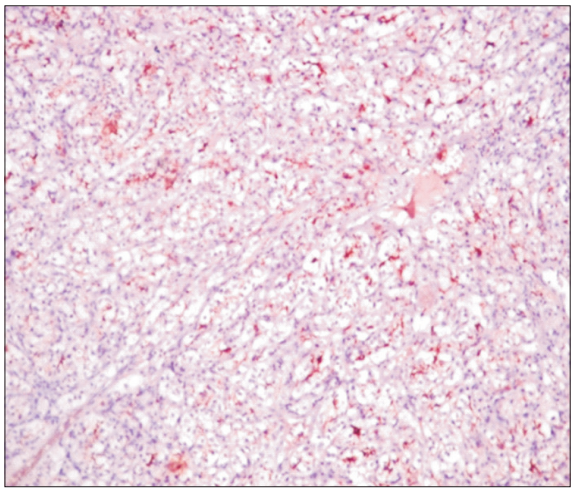

Figure 24: Grade 2 membranous expression of MUC1 in clear cell RCC Nuclear grade -1 (100x)

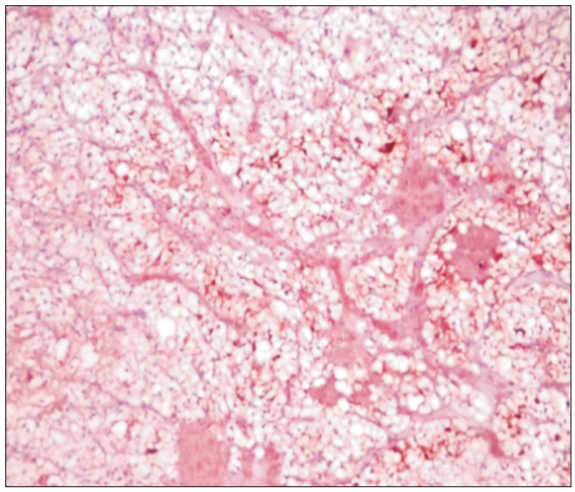

Figure 25: Grade 3 membranous expression of MUC1 in clear cell RCC Nuclear grade -2 (100x)

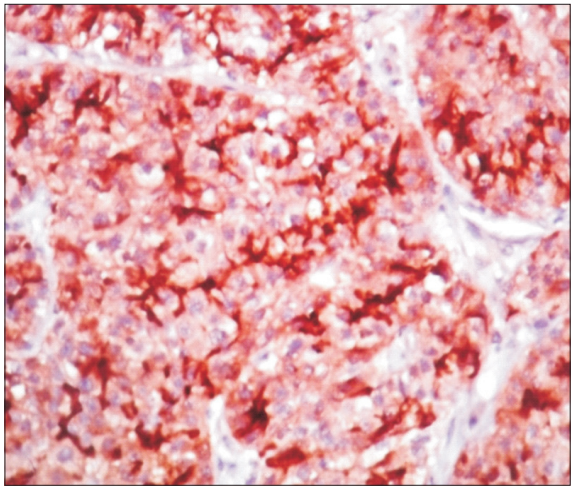

Figure 26: Grade 5 cytoplasmic and membranous expression of MUC1 in clear cell RCC Nuclear grade -3 (400x)

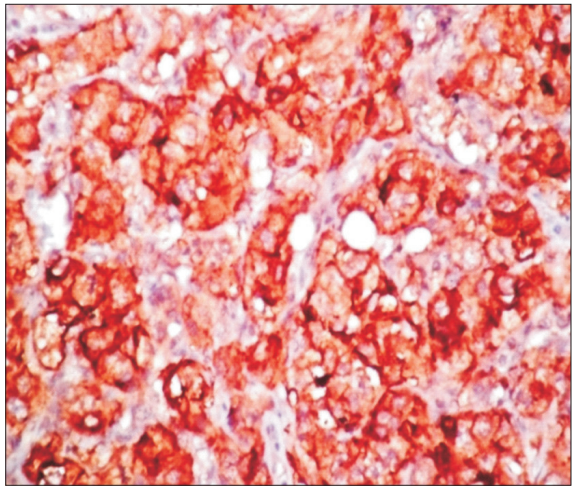

Figure27: Grade 6 cytoplasmic and membranous expression of MUC1 in clear cell RCC Nuclear grade -4(400x)

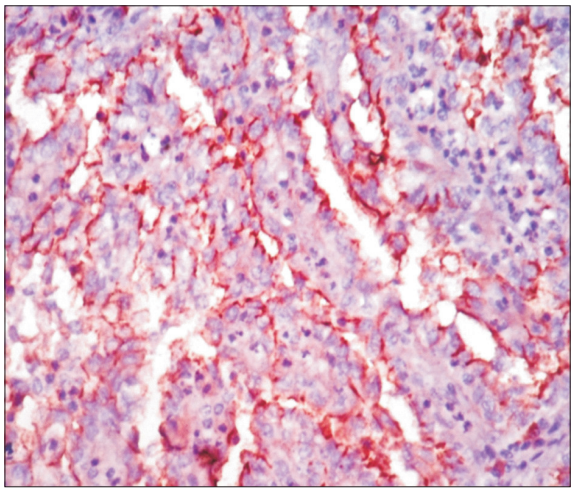

Figure 28: Grade 4 membranous expression of MUC1 in papillary RCC Nuclear grade -3 (100x)

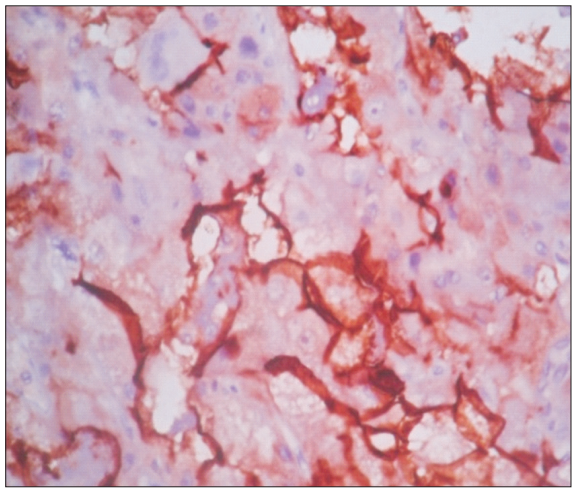

Figure 29: Grade 4 cytoplasmic and membranous expression of MUC1 in chromophobe RCC Nuclear grade -3 (400x)

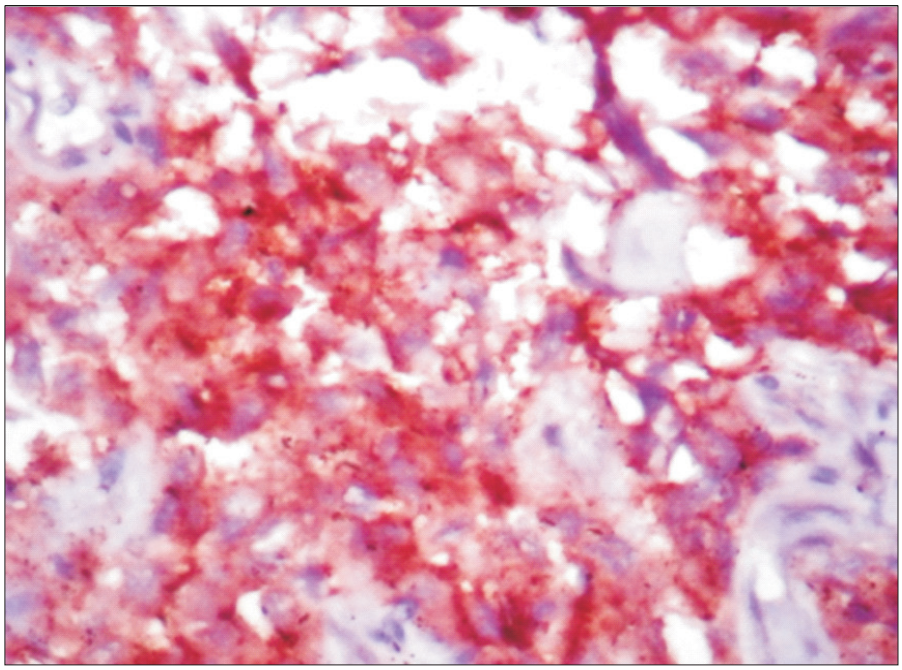

Figure 30: Grade 5 cytoplasmic and membranous expression of MUC1 in unclassified RCC Nuclear grade -4 (400x)

DISCUSSION

DISCUSSION

Among urological malignancies, RCC is the most lethal malignancy. The incidence of RCC is increasing every year at a rate of 2 to 4%.[3] One third of the patients present at the stage of metastasis. In patients with localised disease, surgery is thought to be curative but 30 to 40 % of them develop metastasis during followup.[138] This tumour is highly resistant to radiotheraphy and chemotheraphy.

Prognosis of this tumour is mainly dependant on nuclear grading and staging. It is not always possible to predict the prognosis with these factors.[5,9] RCC pathogenesis is not clear, behaviour and prognosis were difficult to be predicted. Postoperative treatment should be started at the most early in aggressive tumours to prevent metastasis.[132]

Cellular proliferation, apoptosis, metastatic spread are also predictive factors for biological and tumour progression.[6,8] Cellular proliferation rate is evaluated by using Ki67. Apoptosis degree in tumour can be measured by detecting mutated P53 antigen.[6] Metastatic spread can be predicted by measuring the expression of MUC1 in RCC.[8] Combining the expression of P53, Ki67 and MUC1 with nuclear grade will be more effective in predicting the prognosis of this tumour.

In the present study, clinicopathological evaluation was done in 52 cases of RCC. Immunohistochemical study was done for randomly selected 40 cases. An attempt was made to assess the significance of expression of P53, Ki67 and MUC1 in RCC cases. Correlation of expression of P53, Ki67 and MUC1 with nuclear grade was also made.

In this study, among 52 cases of renal cell carcinoma, maximum 36 (69.23 %) cases were of clear cell RCC and 2nd maximum 7 (15.3 %) cases were of papillary RCC. Minimum cases of 2(3.8%) were of chromophobe RCC. This was found to be correlating with the following studies.

TABLE 52: COMPARISON OF TYPES OF RENAL CELL CARCINOMA

TYPES	Ingrid Hodorovaa et al[133]	TimJ. Dudderidge et al[139]	Petard et al[140]	Zou H et al[141]	Current study
CLEAR CELL RCC	69%	66.5%	87.7%	67.5%	69.2%
PAPILLARY RCC	11.9%	13%	9.7%	9.6%	15.3%
CHROMOPHOBE RCC	7.1%	2.8%	2.5%	12.3%	3.8%
RCC, UNCLASSIFIED	9.5%	15%	-	1.8%	11.5%

In all these studies, clear cell RCC is found to be the commonest type of RCC and the second commonest is papillary RCC.

In current study, the age of renal cell carcinoma patients ranged from 27 years to 80 years with the mean age of 53 years. The highest incidence of renal cell carcinoma occurred in 41 to 50 year age group. This is in concurrence with the study done by Farahnaz Noroozinia et al[137] who observed a mean age of 56 ± 6 years with a range from 19 to 86 years.

TABLE 53: COMPARISON OF MEAN AGE OF RENAL CELL CARCINOMA

MEAN AGE	STUDIES
56 ± 6yrs	Farahnaz Noroozinia et al[137]
58yrs(18-82)	Robert et al[142]
53 yrs(26-75)	Sakineh Amouian et al[6]
53 yrs(27-80)	Current study

Also observed that 50% of renal cell carcinoma occurred in age less than or equal to 50 years. According to Pierre. I. Karakiewicz et al,[143] the risk of RCC-specific mortality was lowest among patients younger than 50 years. Young patients are at 87% lower risk for RCC-specific mortality than those aged 50–75 years. The effect of age shows

prognostic significance; follow-up and secondary treatments should be adjusted according to the age of the patient.[143]

In the study done by Kyungeun Kim et al,[144] age range in clear cell carcinoma is 28 to 86 years and in current study, age range is 27-77 years. In the study done by Xavier Leroy et al,[145] mean age in papillary RCC is 60 years (range, 27 to 81 y) and in current study, mean age is 52 yrs(range,38 -68y).

According to study done by N. K. Bhattacharyya et al,[146] incidence of renal cell carcinoma among males was 73.3% and females was 26.6% with a M:F ratio of 2.7:1. In concurrence with the above study, a significant male predominance who accounted for 73.1% of RCC cases and women accounted for 26.9% was observed in current study. The male: female ratio observed in current study was 2.7:1. The similar sex distribution was observed in many studies. From all these studies males have increased risk of RCC when compared to females.

TABLE 54: COMPARISON OF SEX OF RENAL CELL CARCINOMA

SEX	N. K. Bhattacharyya et al[146]	TimJ. Dudderidge et al[139]	Current study
Male	73.3%	68.2%	73.1%
Female	26.6%	31.8%	26.9%
Ratio	2.7:1	2.1:1%	2.7:1

Right sided tumors were more common than left sided tumors in current study and this is in concurrence with the study done by Devendar Katkooriet al.[147] There is no significant difference between right and left side tumors in terms of disease specific survival.

TABLE 55: COMPARISON OF LATERALITY OF RENAL CELL CARCINOMA

STUDIES	RIGHT SIDE	LEFT SIDE	BILATERAL
Ning Yi Yap et al[148]	48.3%	50%	0.7%
DevendarKatkoori et al[147]	69%	31%	-
Current study	67%	33%	-

In current study, 38(73.1%) tumors were size more than 5 cm. 14(26.9%) tumors were size less than equal to 5 cm. This was in concurrence with the study done by Farahnaz Noroozinia et al;[137] in this

study among 64 cases, 48(75%) tumors were size more than 5 cm. In both studies most of the tumors were more than 5 cm.

In this study of 52 cases median tumor diameter is 7.5 cm. This is in concurrence with the study done by Lorenzo Tosco et al[149] where the median tumour diameter was 7.5 cm . In the study done by Jean-Jacques Patard et al,[150] median tumour diameter was 6 cm.

In this study 4 cases showed ureter involvement and the percentage was 7.6%.according to Farahnaz Noroozinia et al[137] percentage of ureter involvement was 6.3% .

In this study 10 cases showed renal vessel invasion and the percentage was 19.2%. This was in concurrence with following studies.

TABLE 56: COMPARISON OF RENAL VESSEL INVASION OF RENAL CELL CARCINOMA

STUDIES	PERCENTAGE OF INVASION
Current study	19.2%
Parekh DJ et al[151]	22%
FarahnazNoroozinia et al[137]	12.5%

In this study, regional lymph nodal involvement present among 4(7.7%) cases and this was not in concurrence with the following studies.

TABLE 57: COMPARISON OF LYMPH NODE INVOLVEMENT OF RENAL CELL CARCINOMA

STUDIES	PERCENTAGE OF LYMPHNODE INVOLVEMENT
Chunwoo Lee et al[152]	4%
Giacomo Novara et al[153]	4%
K Fujita et al[136]	17.7%
Current study	7.7%

Among the 52 cases of renal cell carcinoma, distant metastasis present among 3(5.8%). In all following studies distant metastasis was found to be less than 10%.

TABLE 58: COMPARISON OF METASTASIS OF RENAL CELL CARCINOMA

STUDIES	PERCENTAGE OF METASTASIS
Current study	5.8%
Alexander Laird et al[138]	7.1%
Chunwoo Lee et al[152]	8.5%
Giacomo Novara et al[153]	6%

Among the 52 cases of renal cell carcinoma, maximum cases were presented at T1 stage and 2nd maximum cases were presented at T3 stage. Minimum cases were presented at T4 stage. This was correlating

with the study done by Chunwoo Lee et al.[152] In the study done by Lorenzo Tosco et al,[149] T3 is the most common stage of presentation.

TABLE 59: COMPARISON OF T stage AT PRESENTATION IN RENAL CELL CARCINOMA

T stage at presentation	Lorenzo Tosco et al[149]	Current study	Chunwoo Lee et al[152]
T1	25 (23%)	18(34.6%)	71%
T2	24 (22%)	13(25%)	12.5%
T3	46 (42.2%)	15(28.8%)	15.5%
T4	14(12.8%)	6(11.5%)	1.8%
Total	109	52	

In this study most of the clear cell RCC were T1 stage at presentation and least stage of presentation was T4 stage. This was in concurrence with the study done by Thomas Weber et al.[154]

In several studies stage1is the common stage of presentation and stage 4 is the least common stage of presentation which was in concurrence with this study.

In this study among the 52 cases of renal cell carcinoma, maximum 17(32.7%) cases were of grade 3 and 2nd maximum 16(30.8 %) cases were of grade 2. This was correlating with the study done by

N.K.Bhattacharyyaet al,[146] in which maximum cases were of grade 3 and grade 2.

TABLE 60 : COMPARISON OF NUCLEAR GRADE OF RENAL CELL CARCINOMA

Nuclear grade	Sakineh Amouian et al[6]	N.K.Bhattacharyya et al [146]	Jean-Jacques Patard et al[150]	Current study
G1	30%	10%	20.5%	17.3%
G2	43%	36.6%	36.7%	30.8%
G3	16.9%	36.6%	34.4%	32.7%
G4	10%	16.6%	8.4%	19.2%

In this study, most of the clear cell RCC cases were seen in in grade 2 and 3. This was correlating with the study done by N. K. Bhattacharyya et al.[146] Most of the papillary RCC were of grade 2. This was correlating with the study done by Xavier Leroy et al.[145]

KI67

In this study, 72.5% of tumors were positive for ki67. In the study done by Sakineh Amouian et al,[6] out of 30 tumor studied, 20(66.6%) were positive for ki67. In the study done by Brett Delahunt et al,[155]206 cases were studied and ki67 expression was detected in 83%. In 1239 cases studied by Kai Zheng et al,[132], ki67 was detected in 47.7%.

TABLE 61: COMPARISON OF KI67 POSITIVITY OF RENAL CELL CARCINOMA

STUDIES	PERCENTAGE OF KI67 POSITIVITY
SakinehAmouian et al[6]	66.6%
Brett Delahunt et al[155]	83%
Kai Zheng et al[132]	47.7%
Current study	72.5%

In grade 1 tumours, range of ki67 labelling index was 2-14%. In grade 2, range of ki 67 labelling index was 4-36%. This was in concurrence with the study done by Brett Delahunt et al.[155]

In this study, range of ki67 labelling index in clear cell RCC was 0 to 69%.This is in concurrence with the study done by Matthew H. T. Bui et al;[156] in his study, Ki67 labelling ranged from 0% to 60% and upto 64% in the study done by Wafaa Helmy et al[7].

In high grade tumors of clear cell RCC, ki67 labelling index was 30-69%. This was concurrence with the study done by Wafaa Helmy et al.[7]

In this study there is an increase in proliferative index of ki67 with increase in nuclear grade. This was similar to the studies done by Peter K

Wong et al,[157]Matthew H. T. Bui et al,[156]Kai Zheng et al,[132] Sakineh Amouian et al,[6] Arnold B. Gelb et al.[158]

There is no significant correlation between ki67 expression and tumor types. This was similar to the study conducted by Peter K. Wong et al.[157]

In current study, size of the tumor failed to show a correlation with Ki-67. This was in concurrence with the study done by Peter K. Wong et al.[157]

In the study done by Minna Kankuri et al,[134] Ki67 expression was correlated with stage .This was in concurrence with this study.

P53

In this study, 25% of cases were positive for p53 expression. In the study done by Farahnaz Noroozinia et al,[137] thirteen of 64 (20.3%) cases were P53 positive. In the study done by Sakineh Amouian et al,[6] 13 out of 30 tumors(43.3%) were positive for p53. There is variable expression of p53 in different studies.

In the study done by Ingrid Hodorovaa et al[133] and Farahnaz Noroozinia et al,[137] p53 expression was found to be higher in other

types of RCC than in the clear cell type of RCC; similar findings were also observed in current study.

Papillary RCC has high p53 expression when compared to clear cell RCC. This was correlating with the study done by Farahnaz Noroozinia et al;[137] in their study, 94.8% of clear cell RCC were negative for p53 and 53.8% of papillary RCC were positive for p53 and their study has a positive correlation between p53 expression and histological type with a p value of <0.05 which was similar to the finding observed in the current study.

In this study 20 (50%) of RCC cases were not immunoreactive for p53.25% of RCC expressed p53 in the range of 1-10%. Maximum expression of p53 was seen in 2.5% of cases. In the study done by Ingrid Hodorovaa et al,[135] 42 RCC cases were studied and 66% were not immunoreactive for p53 and 19% of RCC expressed p53 in the range of 1-10% and maximum expression of p53 was seen in 4.7% of cases.

The expression of p53 with nuclear grade in current study is found to correlate with the study done by Farahnaz Noroozinia et al.[137] In both the studies there is no correlation between p53 expression and nuclear grade.

TABLE 62: COMPARISON OF P53 EXPRESSION WITH NUCLEAR GRADING OF RENAL CELL CARCINOMA

Farahnaz Noroozinia et al[137]			CURRENT STUDY	
Nuclear grade	P53 positive	P53 negative	P53 positive	P53 negative
Grade1	0	100%	0	100%
Grade2	26%	74%	27%	73%
Grade3	25%	75%	25%	75%
Grade4	0	0	40%	60%
P value	0.1		0.3	

In current study, there is no correlation between age, gender, size with P53 expression. This is in concurrence with the study done by Farahnaz Noroozinia et al.[137]

MUC1

In this study more than 90% of cases of RCC cases were immunoreactive for MUC1 and this is in concurrence with the study done by Cord Langner et al.[135]

In this study 85%cases of clear cell RCC were positive for MUC1 expression. In the study done by Xavier Leroy et al[8] MUC1 expression was found in all Clear cell RCCs.

In papillary RCC, out of 7 cases, 6 were positive for MUC1 expression. The cases which are positive are all type 1 tumors and only one type 2 tumor studied which was negative for MUC1. This was in concurrence with the study done by cord langner et al.[135]In his study in papillary carcinomas, MUC1 immunoreactivity was found in 12/12 (100%) type 1 tumours and also in concurrence with study done by Xavier Leroy et al.[145]

In this study, all 2 chromophobe cases showed positive MUC1 expression; in the study done by cord langner et al,[135] 20/22(91%) showed positivity.

In all the types of RCC, expression of MUC1 was more than 50% in current study. This was in correlation with the study done by Cord Langner et al;[135] in their study, MUC1 immunoreactivity of more than 50% of tumor cells was found in 68/133 (51%) conventional, 15/22 (68%) chromophobe, and 11/20 (55%) papillary RCC.

According to study done by K Fujita et al,[136] 51 cases of RCC were studied for MUC1 expression. In their study, K Fujita et al[136] observed a positive correlation of nuclear grade with MUC1 expression with a p value of <0.05 which found to be correlated with current study with a p value of 0.02.

SUMMARY

SUMMARY

- From august 2011 to august 2013, a total of 31,237 specimens were received for histopathological examination in the institute of pathology, Madras Medical College.
- Among this, 52 were nephrectomy specimens done for RCC.
- Of this, 36 were clear cell RCC, 8 were papillary RCC, 2 were Chromophobe RCC, 6 were unclassified RCC.
- Clear cell RCC was the most common histological type**.**
- Most common nuclear grade was grade 3 (32.8%) followed by grade 2 (32.7%).
- The mean age of Renal cell carcinoma in this study was 53 years.
- The youngest age of presentation was 27 years and the oldest age of presentation was 80 years.
- The incidence of RCC was comparatively higher in the age group of 41-50 years.
- In this study there was an overall male preponderance.

- Right sided tumors were more common than left sided tumors accounting for 67.31%.
- The maximum dimension of most of the RCC were more than 5 cm (38 cases, 73.1%).
- The median diameter of the tumor is 7.5 cm.
- Capsular infiltration was present in 32.7%.
- Perinephric tissue involvement was present in 25%.
- Gerota's fascia involvement was present in 9.6%
- Ureter invasion was present in 7.7%.
- Renal vessel invasion was present in 19.2%.
- Adrenal involvement was present in 7.7%.
- 7.7% of the patients had regional lymphnodal involvement.
- 5.8% of the patients had distant metastasis at the time of presentation.
- Maximum cases of renal cell carcinoma presented in T1bstage (21.6%) and T3a stage (21.6%).
- Most common stage at the time of diagnosis was stage 1(34.6%)

- Among the total 40 cases, 29 cases showed positivity for KI67, accounting for 72.5%.
- No statistical significance was found between the histological type and KI67 expression.
- KI67 expression was high in nuclear grade 4 tumors (9 cases,90%)
- The association was found to be statistically significant between nuclear grade and KI67 expression
- Nuclear grade 3 tumors had high KI67 labeling index and the mean LI was 36.9% for grade 3 tumors.
- No statistical significance was found between the age and KI67 expression.
- The association between sex and KI67 expression was not statistically significant.
- The association between size of the tumor and KI67 expression was not statistically significant.
- The association was found to be statistically significant between stage and KI67 expression.

- Among the total 40 cases, 10 cases showed positivity for P53, accounting for 25%.
- 2.5% of cases had high level of P53 expression.
- The association was found to be statistically significant between histological type of the tumor and P53 expression.
- No statistical significance was found between nuclear grade and P53 expression.
- No statistical significance was found between age and P53 expression.
- The association between sex and P53 expression was not statistically significant.
- The association between size of the tumor and P53 expression was not statistically significant.
- The association between stage at presentation and P53 expression was not statistically significant.
- Among the total 40 cases, 35 cases showed positivity for MUC1, accounting for 87.5%.
- Maximum cases (13 cases,32.5%) had MUC1expression in the range of 75-90%.

- The association between histological type and MUC1 expression was not statistically significant.
- All grade 4 tumors had more than 75% MUC1 expression.
- The association was found to be statistically significant between nuclear grade and MUC1 expression.
- No statistical significance was found between age and MUC1 expression.
- No statistical significance was found between sex and MUC1 expression.
- No statistical significance was found between size of the tumor and MUC1 expression.
- The association was found to be statistically significant between stage at presentation and MUC1 expression.

CONCLUSION

CONCLUSION

This study was hospital-based and may not represent the true incidence of the disease in the community. 52 cases of Renal Cell Carcinoma were studied in the Institute of Pathology from august 2011 to august 2014. The mean age of occurrence of RCC is 53 years with predominance in males. The most common histological type noted was clear cell RCC, with majority of the cases exhibiting nuclear grade 3.

There is association of ki67 expression with nuclear grade and stage. There is no correlation between P53 and nuclear grade. The expression of P53 is found to be associated with histological types. There is association of MUC1 expression with nuclear grade and stage.

The combined detection of p53, Ki67, MUC1 expressions, which are superior to single marker along with nuclear grade and stage, could be used to significantly improve the accuracy in predicting the prognosis of RCC patients.

ANNEXURES

ANNEXURE – I

PROFORMA

Case number : Name :

HPE number : Age :

IP number : Sex :

Clinical history :

Clinical diagnosis :

Imaging :

Type of procedure done:

Nature of specimen :

GROSS

Tumour location :

Tumour size :

Tumour configuration : capsular infiltration:

Renal pelvis: perinephric tissue:

Renal vessels: Gerota's fascia:

Ureter: Adjacent kidney :

Adrenal gland:

Lymph Nodes :

MICROSCOPY

Histological type : Furhman nuclear grade : G1 / G2 / G3 / G4

Renal pelvis:

Capsular infiltration: present/ absent

Perinephric tissue: free/ involved

Gerota's fascia: free/ involved

Renal artery invasion: present/ absent

Renal vein invasion: present/ absent

Ureter : free/ involved

Adrenal: free/ involved

Lymph nodes-total nodes dissected and number of nodes involved

Distant metastasis :

TNM staging :

IMMUNOHISTOCHEMISTRY

Ki67 : positive /negative
% of tumour nuclei showing reaction

P53: positive /negative
% of tumour nuclei showing reaction and grade

MUC1: positive /negative
% of tumour cells showing membranous/cytoplasmic positivity and grade

ANNEXURE – II

WHO histological classification of tumours of the kidney

Renal Cell Tumors

Clear cell renal cell carcinoma

Multilocular clear cell renal cell carcinoma

Papillary renal cell carcinoma

Chromophobe renal cell carcinoma

Carcinoma of the collecting ducts of Bellini

Renal medullary carcinoma

Xp11 translocation carcinomas

Carcinoma associated with neuroblastoma

Mucinous tubular and spindle cell carcinoma

Renal cell carcinoma, unclassified

Papillary adenoma

Oncocytoma

Metanephric tumours

Metanephric adenoma

Metanephric adenofibroma

Metanephric stromal tumour

Nephroblastic tumours

Nephrogenic rests

Nephroblastoma

Cystic partially differentiated nephroblastoma

Mesenchymal tumours

Occurring Mainly in Children

- Clear cell sarcoma
- Rhabdoid tumour
- Congenital mesoblastic nephroma
- Ossifying renal tumour of infants

Occurring Mainly in Adults

- Leiomyosarcoma (including renal vein)
- Angiosarcoma
- Rhabdomyosarcoma
- Malignant fibrous histiocytoma
- Haemangiopericytoma
- Osteosarcoma
- Angiomyolipoma
  - Epithelioid angiomyolipoma
- Leiomyoma
- Haemangioma
- Lymphangioma
- Juxtaglomerular cell tumour
- Renomedullary interstitial cell tumour

Schwannoma

Solitary fibrous tumour

Mixed mesenchymal and epithelial tumours

Cystic nephroma

Mixed epithelial and stromal tumour

Synovial sarcoma

Neuroendocrine tumours

Carcinoid

Neuroendocrine carcinoma

Primitive neuroectodermal tumour

Neuroblastoma

Phaeochromocytoma

Haematopoietic and lymphoid tumours

Lymphoma

Leukaemia

Plasmacytoma

Germ cell tumours

Teratoma

Choriocarcinoma

Metastatic tumors

ANNEXURE – III

TNM classification of renal cell carcinoma

T – Primary Tumour

TX- Primary tumour cannot be assessed

T0 -No evidence of primary tumour

T1 -Tumour 7 cm or less in greatest dimension, limited to the kidney

T1a- Tumour 4 cm or less in greatest dimension, limited to the kidney

T1b -Tumour more than 4 cm but not more than 7 cm in greatest dimension, limited to the kidney

T2 - Tumour more than 7 cm in greatest dimension, limited to the kidney

T2 a - Tumour more than 7 cm but less than 10 cm in greatest dimension, limited to the kidney

T2 b - Tumour more than 10 cm in greatest dimension, limited to the kidney

T3 - Tumour extends into major veins or perinephric tissues but not into adrenal gland and not beyond Gerota fascia

T3a - Tumour grossly extends into the renal vein or its segmental (muscle containing) branches, or tumor invades perirenal and/or renal sinus fat but not beyond Gerota's fascia.

T3b - Tumour grossly extends into vena cava below diaphragm

T3c - Tumour grossly extends into vena cava or its wall above diaphragm

T4 - Tumour directly invades beyond Gerota fascia(including contiguous extension into the ipsilateral adrenal gland)

N – Regional Lymph Nodes

NX - Regional lymph nodes cannot be assessed

N0 - No regional lymph node metastasis

N1- Metastasis in regional lymph node

M – Distant Metastasis

MX - Distant metastasis cannot be assessed

M0 - No distant metastasis

M1 - Distant metastasis

Anatomic Stage/ prognostic groups

Stage I	T1 N0 M0
Stage II	T2 N0 M0
Stage III	T3 N0 M0
	T1, T2, T3 N1 M0
Stage IV	T4 N0, N1 M0
	Any T Any N M1

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MASTER CHART

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HPE NO	AGE	SEX	SITE	SIZE	CI	PI	GF	UI	RV	RA	AI	LN	MT	Tst	ST	HT	NG	KI67 LI	KI67 +/-	P53 LI	p53G	p53 +/-	MUC1 %	MUC1 G	MUC1+/-
6412/11	46	M	L	10	P	A	A	P	P	A	A	A	A	T3a	3	1	3	50	POS	40	2+	POS	95	6	POS
6834/11	60	M	R	8	P	P	A	A	A	A	A	A	A	T3a	3	4	3								
7277/11	43	M	L	4	P	P	P	A	A	A	P	A	A	T4	4	1	3	40	POS	4	1+	NEG	76	5	POS
7462/11	55	M	L	7	A	A	A	A	A	A	A	A	A	T1b	1	1	1	5	NEG	0	0	NEG	23	2	POS
7870/11	42	F	R	7	A	A	A	A	A	A	A	A	A	T1b	1	1	1	0	NEG	0	0	NEG	8	1	NEG
8363/11	40	M	R	6	A	A	A	A	A	A	A	A	A	T1b	1	2	2	0	NEG	3	1+	NEG	8	1	NEG
8824/11	60	F	L	4	A	A	A	A	A	A	A	A	A	T1a	1	1	1								
8927/11	52	M	R	8	P	P	A	A	P	P	P	A	A	T4	4	1	4	62	POS	0	0	NEG	91	6	POS
9005/11	35	F	R	2	A	A	A	A	A	A	A	A	A	T1a	1	1	2	4	NEG	0	0	NEG	40	3	POS
9151/11	50	M	L	6	A	A	A	A	A	A	A	A	A	T1b	1	1	2	32	POS	73	2+	POS	80	5	POS
9225/11	40	F	L	6	A	A	A	A	A	A	A	P	A	T3b	3	1	3	39	POS	5	1+	NEG	62	4	POS
9319/11	47	M	R	15	P	A	A	A	P	A	A	A	A	T3a	3	4	4	5	NEG	0	0	NEG	76	5	POS
21/2012	68	M	R	5	A	A	A	A	A	A	A	A	A	T1b	1	1	2								
1586/12	50	M	R	5	P	P	P	A	A	A	P	A	A	T4	4	1	3								
1701/12	65	M	R	3	A	A	A	A	A	A	A	A	A	T1a	1	1	1	5	NEG	0	0	NEG	9	1	NEG
1702/12	38	M	L	3	A	A	A	A	A	A	A	A	A	T1a	1	2	1	2	NEG	0	0	NEG	90	5	POS
1986/12	44	M	R	9	P	A	A	A	A	A	A	A	A	T2a	2	1	2	25	POS	0	0	NEG	48	3	POS
3703/12	68	F	L	8	A	A	A	A	A	A	A	A	A	T2a	2	1	2	36	POS	5	1+	NEG	32	3	POS
4698/12	56	M	R	9	P	A	A	A	P	P	A	A	A	T3b	3	1	4	69	POS	0	0	NEG	92	6	POS
5508/12	45	M	R	11	P	A	A	A	A	A	A	A	A	T2b	2	1	2								
6525/12	80	M	R	9	A	A	A	A	A	A	A	A	A	T2a	2	3	2	11	POS	25	2+	POS	21	2	POS
6985/12	57	F	L	10	P	A	A	A	P	A	A	P	P	T3a	4	4	3								
7914/12	40	F	R	9	P	A	A	A	P	A	A	A	A	T3a	3	1	3	56	POS	4	1+	NEG	77	5	POS
8005/12	65	M	R	13	A	A	A	A	A	A	A	A	A	T2b	2	1	2								
9139/12	27	M	R	6	P	P	A	A	A	A	A	A	A	T3a	3	1	2								
9154/12	68	M	L	6	A	A	A	A	A	A	A	A	A	T1b	1	2	2								
9892/12	65	M	R	8	A	A	A	A	A	A	A	A	A	T2a	2	1	1	14	POS	4	1+	NEG	96	6	POS

240/13	50	F	R	5	A	A	A	P	A	A	A	A	A	T1b	1	2	3	39	POS	35	2+	POS	52	4	pOS
477/13	45	F	R	4	P	P	A	A	A	A	A	P	A	T3a	3	2	4	64	POS	0	0	NEG	80	5	POS
821/13	77	M	R	12	A	A	A	A	A	A	A	A	A	T2b	2	1	3								
1068/13	39	M	R	11	A	A	A	P	A	A	A	A	A	T2b	2	1	4	70	POS	80	2+	POS	84	5	POS
2795/13	49	M	L	9	P	P	P	P	P	P	P	A	A	T4	4	4	4	66	POS	0	0	NEG	73	5	POS
5322/13	58	F	R	9	A	A	A	A	A	A	A	A	A	T2a	2	1	3								
6945/13	50	M	R	6	A	A	A	A	A	A	A	A	A	T1b	1	2	2	16	POS	31	2+	POS	63	4	POS
7058/13	65	M	R	9	A	A	A	A	P	A	A	A	A	T3a	3	3	3	16	POS	44	2+	POS	60	4	POS
7071/13	59	M	R	10	P	P	P	A	A	A	A	P	P	T4	4	4	3	9	NEG	0	0	NEG	50	4	POS
7793/13	51	M	R	8	A	A	A	A	A	A	A	A	A	T2a	2	1	2	5	NEG	5	1+	NEG	65	4	POS
8386/13	51	M	R	8	P	P	A	A	A	A	A	A	A	T3a	3	1	4	69	POS	4	1+	NEG	94	6	POS
9029/13	50	M	L	4	A	A	A	A	P	A	A	A	A	T3b	3	1	4	64	POS	54	2+	POS	80	5	POS
9186/13	48	M	R	4	A	A	A	A	A	A	A	A	A	T1a	1	1	1								
9453/13	40	F	R	5	A	A	A	A	A	A	A	A	A	T1b	1	1	2	3	NEG	0	0	NEG	82	5	POS
10350/13	63	M	L	7	A	A	A	A	A	A	A	A	A	T1b	1	2	3	25	POS	5	1+	NEG	40	3	POS
11336/13	52	M	R	6	P	P	A	A	A	A	A	A	A	T3a	3	1	3	56	POS	0	0	NEG	80	5	POS
1313/14	39	M	R	4	A	A	A	A	A	A	A	A	A	T1a	1	1	1	11	POS	0	0	NEG	72	4	POS
2589/14	62	M	L	15	P	P	P	A	A	A	A	A	P	T4	4	1	3	53	POS	0	0	NEG	80	5	POS
2641/14	75	M	L	4	A	A	A	A	A	A	A	A	A	T1a	1	1	2	20	POS	0	0	NEG	9	1	NEG
2773/14	52	M	L	9	A	A	A	A	A	A	A	A	A	T2a	2	1	2	32	POS	0	0	NEG	30	3	POS
3480/14	47	M	R	9	A	A	A	A	A	A	A	A	A	T2a	2	1	3	30	POS	0	0	NEG	87	5	POS
3789/14	31	F	R	6	P	P	A	A	A	A	A	A	A	T3a	3	4	4	72	POS	93	3+	NEG	98	6	POS
4368/14	65	F	R	8	A	A	A	A	A	A	A	A	A	T2a	2	2	4	56	POS	25	2+	POS	91	6	POS
5704/14	62	M	L	10	A	P	A	A	P	P	A	A	A	T3c	3	1	3	30	POS	4	1+	NEG	50	2	POS
5842/14	42	F	R	6	A	A	A	A	A	A	A	A	A	T1b	1	1	1	4	NEG	0	0	NEG	0	0	NEG

KEY TO MASTER CHART

1.	HPE NO	-	HISTOPATHOLOGICAL EXAMINATION NUMBER
2.	M	-	MALE
3.	F	-	FEMALE
4.	R	-	RIGHT
5.	L	-	LEFT
6.	P	-	PRESENT
7.	A	-	ABSENT
8.	CI	-	CAPSULAR INFILTRATION
9.	PI	-	PERINEPHRIC TISSUE INVOLVEMENT
10.	GF	-	GEROTA'S FASCIA INVOLVEMENT
11.	UR	-	URETER INVASION
12.	RV	-	RENAL VEIN INVASION
13.	RA	-	RENAL ARTERY INVASION
14.	AI	-	ADRENAL INVOLVMENT
15.	LN	-	LYMPH NODE INVOLVEMENT
16.	MT	-	METASTASIS
17.	ST	-	STAGE
18.	HT	-	HISTOLOGICAL TYPE

1 - CLEAR CELL RCC

2 - PAPILLARY RCC

3 - CHROMOPHOBE RCC

4 - UNCLASSIFIED RCC

19.	NG	-	NUCLEAR GRADE
20.	LI	-	LABELLING INDEX
21.	POS	-	POSITIVE
22.	NEG	-	NEGATIVE
23.	P53 G	-	P53 GRADE
24.	MUC1 G	-	MUC1 GRADE